EFFECT OF DEXAMETHASONE TREATMENT ON PAI-1 PRODUCTION BY HUMAN LUNG FIBROBLASTS

Kimiko TAKAHASHI1), Katsuyuki KOBAYASHI2), Masako MITSUI2), Tomotada ODAKA2), Hiroyuki NAKAMURA2), Takeshi MATSUOKA2)

1) Department of Nutrition, Aizu University, Aizu-Wakamatsu, Fukushima, Japan.
2) Fifth Department of Internal Medicine, Tokyo Medical University, Kasumigaura Hospital, Ami, Ibaraki, Japan.

Abstract Pulmonary fibroblasts actively secrete PAI-1 at injured sites. KiF fibroblasts with the deletion allele of the PAI-1 promoter gene produced and secreted larger amounts of PAI-1 than WI-38 fibroblasts with the insertion allele. The effects of dexamethasone on PAI-1 expression after stimulation with TNFα were examined using these two human lung fibroblast lines. Dexamethasone initially reduced TNFα-induced NF-κB translocation, PAI-1 mRNA expression and PAI-1 secretion. The effects in KiF appeared earlier than in WI-38. However, longer treatment with dexamethasone eventually led to larger PAI-1 secretion than that observed in TNFα-unstimulated cells. These results suggest that the polymorphism of the PAI-1 promoter gene regulates not only PAI-1 production but also the response to dexamethasone.

Key Words: PAI-1, fibroblasts, dexamethasone

Introduction Pulmonary fibroblasts proliferate in damaged alveolar spaces, and they actively secrete plasminogen activator inhibitor-1 (PAI-1)1–3). Large amounts of urokinase-type plasminogen activator (uPA), a fibrinolytic enzyme, are present in healthy pulmonary tissue compared with other tissues4,5). We previously found that the sources of uPA are microvascular endothelial cells, alveolar epithelial cells and macrophages6–9). Lung fibroblasts produce more PAI-1 than uPA6,7). Normal fibrinolytic activity within the alveolar space is suppressed in fibrotic lung diseases characterized by excessive and irregular deposition of extracellular matrix proteins such as collagen, in part due to increased levels of PAI-11,2,3,10,11). Increased PAI-1 activity is a hallmark of tissue and organ fibrosis12). Some studies have shown that inhibition of the plasminogen system by PAI-1 induces the development of pulmonary fibrosis in animals11,13).

PAI-1, a serine protease inhibitor belonging to the serpin family, is a single-chain 45 to 50-kDa glycoprotein secreted by many cell types. It binds to uPA in order to modulate plasmin activation. The functions of uPA are thought to include both degradation of the extracellular matrix and activation of metalloproteinases or growth factors14,15). The fibrinolytic activity on the cell surface is related to cell migration and tissue remodeling by dissolution of the extracellular matrix and basement membrane16,17). PAI-1 also leads to cell detachment by deactivation and internalization of integrins that are bound to the uPA receptor (uPAR)18).

A single-base-pair insertion/deletion polymorphism in the upstream promoter of the PAI-1 gene that yields alleles containing either 4 or 5 consecutive guanines (4G or 5G) was recently found19,20). The insertion/deletion point is 675 base pairs upstream of the transcription initiation site of PAI-1. It has been reported that this poly-
morphism site is the binding point for NF-κB families[19]. The frequency of the PAI-1 promoter deletion allele (4G) in the general population is about 51 to 54%, and it produces six-fold more PAI-1 than the insertion (5G) allele[20]. The relationship between this polymorphism and cardiovascular diseases has been investigated, and it probably plays a role in the pathophysiological processes of certain cardiovascular disorders[21,22]. PAI-1 is also a key substance in diseases such as keloids, interstitial nephritis, coronary heart diseases, hepatic cirrhosis and obesity[1,10,21-24]. In these tissues, the increase in PAI-1 causes collagen accumulation or low blood fluidity, which may directly influence the pathological progress[21-23]. Although there may be specific characteristics in the early events associated with the development of fibrosis in different tissues, the fundamental mechanisms resulting in excessive deposition of extracellular matrix proteins are apparently similar. However, it is still unclear whether this PAI-1 gene polymorphism contributes to the progression of pulmonary fibrosis.

In the sputum in pulmonary diseases such as cystic fibrosis, asthma and COPD, the amounts of inflammatory cytokines and PAI-1 are higher than those in healthy subjects[25]. It is also known that TNFα induces PAI-1 production[26]. Corticosteroids, such as dexamethasone, are routinely used for the treatment of patients with pulmonary fibrosis, though their therapeutic effects depend on the pathological type of disease[27,28]. In this study, we used two strains of human lung fibroblasts: a line isolated from human lung tissue (KiF), in which the PAI-1 promoter contained the deletion allele (4G), and the human lung fibroblast line WI-38, which contained the corresponding insertion allele (5G). After these cells were pre-stimulated with TNFα, the effects of dexamethasone on PAI-1 production were examined.

Materials and Methods

Materials

Collagenase, EDTA, KCl, MgCl₂, glycerol, glycine, NP-40, HEPES, chloroform, isopropanol, Tris, acrylamide, N,N'-methylene-bis(acrylamide), ammonium peroxodisulfate, acetic acid, boric acid, agarose, ethanol, ribollavin, ethidium bromide, dexamethasone, phenylmethylsulfonyl fluoride (PMSF), leupeptin, Tween-20, TritonX-100, WST-8 (cell counting kit), non-fat dry milk, and dithiothreitol (DTT) were from Wako Pure Chemicals (Tokyo, Japan); bovine serum albumin (BSA) was from Boehringer (Indianapolis, IN); aprotinin and urokinase were from Mitsubishi Pharm (Osaka, Japan); medium 199 (M199) and minimum essential medium (MEM) were from Sigma Aldrich (St Louis, MO); fetal calf serum (FCS), trypsin, TRIzol®, DNA ladder, penicillin-streptomycin mixture and Fungizone® were from Invitrogen (Carlsbad, CA); tumor necrosis factor-alpha (TNFα) was from Genzyme (Cambridge, MA); 60 and 90 mm-diameter plastic culture dishes, 96-well plates, were from Becton Dickinson Labware (Franklin Lakes, NJ); 6-well plates were from Costar (Corning, NY); 10-kDa MW cut off centrifugal filter tubes were from Millipore (Bedford, MA); RT-PCR beads, nitrocellulose membrane for Western blotting (Hybond ECL), Hyperfilim and enhanced chemiluminescence (ECL) detection reagents were from Amersham Pharmacia Biotech (Buckinghamshire, UK); anti-human PAI-1 antibody, spectorozyme UK were from American Diagnostica (Stanford, CT); PAI-1 ELISA assay kit was from Hyphen BioMed (Rue d’Ergny, France); nitrocellulose membrane for EMSA test (Biodyne B) was from Pall (Pensacola, FL) and EMSA detection kit was from Panomics (Redwood City, CA); Genomic DNA extraction kit was from Macherey-Nagel (Düren, Germany); restriction enzyme Bsl I was from New England Biolab (Beverly, MA).

Cells

A small section of human lung was obtained within 1–2 mm of the periphery of a normal region of lung in a patient undergoing resection for solitary lung tumor with permission from the Ethics Committee of Tokyo Medical University. Surgery was performed by the Surgical Service of Kasumigaura Hospital, Tokyo Medical University (Ibaraki, Japan). After excision, the lung tissue was treated for 5 min with sterilized 10% povidone-iodine (Meiji Pharmaceutical, Tokyo, Japan) and was rinsed repeatedly with PBS. Tissue was collected from an...
Dexamethasone regulates PAI-1 in lung fibroblasts

area up to 2 mm below the pleurae. The peripheral lung tissue was then minced and digested with 0.1% collagenase in a 37°C shaking water bath. After 30 minutes of digestion, the residue was filtered through 100-μm stainless meshes, and M199 with 10% calf serum to neutralize enzyme activity was added to the filtrate. After washing the cell pellet by centrifugation, alveolar macrophages were removed based on differences in cell adhesiveness. Cells were suspended in M199 supplemented with 10% FCS and were incubated at 37°C in a 5% CO₂ incubator. After an hour of incubation, macrophages were removed by collecting non-adherent cells, and the cells were seeded into dishes, and cultivated in M199 containing 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml fungizone.

Pulmonary fibroblasts, which were long-shaped, non-epithelial cells, were obtained from only one patient. The fibroblasts grew rapidly and actively than the cells obtained from the other patients, and designated KiF. KiF in passages 6–11 were used for each experiment.

The human lung fibroblast cell line WI-38 (IFO50075) was obtained from the Japan Health Science Foundation (Osaka, Japan) and was cultivated with MEM containing with 10% FCS and antibiotics.

Cell Counts

KiF or WI-38 cells were suspended in each growth medium containing with 10% FCS, and were seeded into 96-well plates (5 x 10² cells/well) and cultivated for 24 h to allow cells to attach to the plate, and medium was replaced at 24 and 96 h after inoculation. At 24, 48, 96 and 144 h after seeding, 10 μl of WST-8 was added to each well and plates were incubated at 37°C for 1 h. Absorbance at 405 nm was measured with a microplate reader (3550-UV; Bio-Rad, Tokyo, Japan). Growth was expressed in terms of absorbance after subtracting the absorbance obtained from the same amount of medium (without cells) treated as described above.

Polymorphism of PAI-1 promoter region

Genomic DNA was extracted from each KiF and WI-38, and the promoter region of the human PAI-1 gene was amplified using the PCR method. The set of primers specific for human PAI-1 promoter region²⁹ from which an amplified fragment of 98-bp was obtained, considered of a forward primer with a nucleotide sequences of 5’-CAC AGA GAG AGT CGG CCA GGT-3’ and a reverse primer with a nucleotide sequence of 5’-CCA ACA GAG GAC TCT TGG TCT-3’. PCR was carried out under the following conditions: 30 cycles at 95.0°C for 1 min, 60.0°C for 1 min and 72.0°C for 2 min; 1 cycle at 72.0°C for 5 min (Thermal Cycler PC-800: Astec; Fukuoka, Japan). The PCR products were digested with the restriction enzyme, Bsl I, at 55°C for 2.5 h, and were separated by electrophoresis on 4.0% agarose-Tris-acetate-EDTA gel and stained with ethidium bromide.

Culture conditions

In order to examine the expression of PAI-1, KiF and WI-38 cells were cultured under the conditions described below.

1) Blank (–): KiF and WI-38 cells were pre-incubated with serum-free M199 and MEM (containing 0.3 mg/ml serine protease-inactivated BSA) at 37°C, respectively. After 4 h of cultivation, media were replaced with fresh media containing with 10% FCS and antibiotics.

2) TNFα stimulation (TNFα): Fibroblasts were cultivated with the serum-free media containing 1 ng/ml TNFα for 4 h. At the end of pre-incubation, the media were replaced fresh media containing 1 ng/ml TNFα.

3) Dexamethasone treatment (TNFα + dex): After 4 h of pre-incubation with 1 ng/ml TNFα, cells were treated with serum-free media containing both with 1 ng/ml TNFα and 500 ng/ml dexamethasone. This dose of dexamethasone was comparable to the pharmacologically effective concentration³⁰,³¹.

After the second medium change, cells were incubated for the periods indicated, and conditioned medium (CM), cell lysate or total RNA was harvested.
Western blotting

After KiF or WI-38 cells stimulated with TNFα were treated with or without dexamethasone for 6 h as described above, CM was collected and the cells were lysed in lysis buffer containing 1% TritonX-100, 0.1% SDS, 1 mM PMSF and 25 mg/ml aprotinin. CM was concentrated to 0.5 ml using a 10-kDa cut off centrifuge filter tube (6 fold). The total protein in each sample was measured by the Lowry method32). Equal amounts of protein samples were size-separated on discontinuous 9% polyacrylamide gels and were transferred to nitrocellulose membranes. Membranes were immersed for 60 min in blocking solution containing 5% non-fat dry milk and 0.1% Tween-20, and were then incubated overnight at 4ºC with mouse anti-PAI-1 antibody. After washing, membranes were incubated for 1 h at room temperature with peroxidase-linked secondary antibody. Blotted proteins were detected using an enhanced chemiluminescence (ECL) detection system.

Electrophoretic mobility shift assay (EMSA)

KiF and WI-38 cells were stimulated as described above, and nuclear extracts were prepared. The cells (10⁷ cells) were lysed (in 10 mM HEPES, pH 7.9, 2 mM MgCl₂, 15 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 10 μg/ml leupeptin, and 0.2% NP-40) on ice. Nuclear proteins were extracted (50 mM HEPES, pH 7.9, 400 mM KCl, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, 0.5 mM PMSF, and 10 μg/ml leupeptin) and the total protein was measured by the Lowry method32).

Gel shifts were performed with 3.0 μg of nuclear extract from KiF or WI-38, pre-incubated at room temperature with 1 μg of poly (dl-dC). After 5 minutes, binding buffer (containing 15 mM HEPES, 30 mM NaCl, 1 mM EGTA, 1 mM DTT, 10% glycerol) and 3'- biotin-labeled NF-κB probe (5'-CCG GTC AGA GGG GAC TTT CCG AGA CT-3') were added to the samples and the resulting mixtures incubated at room temperature for 30 min. In selected samples, either non-specific control probe or unlabeled probe was included in the binding reaction in order to confirm the specificity of DNA-protein interaction.

RT-PCR

KiF and WI-38 cells were stimulated as described above and were lysed by addition of TRIzol®. The total RNA was extracted with chloroform, isopropanol, and ethanol by centrifugation after each addition according to the manufacturer’s instructions. The extracted RNA was dissolved in distilled water, and absorbance was measured with spectrophotometer (UV-1600: Shimazu, Kyoto, Japan) at 260 nm to estimate the amount of total RNA.

PAI-1 and GAPDH sequences were amplified using the one step RT-PCR method with 1 μl of samples, which included the same amount of total RNA from each strain of fibroblasts. The set of primers specific for human PAI-134) from which an amplified fragment of 402-bp was obtained, considered of a forward primer beginning at 19-bp with a nucleotide sequences of 5'-GTC TTT GGT GAA GGG TCT GCT GTG CAC CAT-3' and a reverse primer beginning at 421-bp with a nucleotide sequence of 5'-TGA AAA GTC CAC TTG CTT GAC CGT GCT CCG -3'. The sequences of the GAPDH primer set were based on mammalian consensus sequences derived from conserved amino acid regions, with the 3' end of the primers ending with the first two bases of a rare amino acid from which an amplified fragment of 540-bp was obtained35,36).

RT-PCR was carried out under the following
Dexamethasone regulates PAI-1 in lung fibroblasts

conditions: 1 cycle at 42.0°C for 30 min and 95.0°C for 5 min; 25 cycles at 95.0°C for 1 min, 55.0°C for 1 min and 72.0°C for 4 min; 1 cycle at 72.0°C for 5 min. The PCR products (7 μl) were separated by electrophoresis on 2.0% agarose-Tris-acetate-EDTA gel and were stained with ethidium bromide. The densities of each band were calculated using Scion Image (Frederick, MD) and were normalized by the amount of GAPDH mRNA.

Measurement of PAI-1 antigens

After 3, 6 or 9 h of the treatment with dexamethasone, amounts of PAI-1 antigen in the CM samples of KiF and WI-38 were also determined by the conventional ELISA method according to the manuals of the kit. In brief, samples were added to the plates coated with monoclonal mouse anti-human PAI-1 antibody to allow binding with the immobilized antibody for 1 h at room temperature. Peroxidase-conjugated monoclonal mouse anti-human PAI-1 antibody was added to each well to react with the bound PAI-1 antigen for at room temperature. After 1 h of incubation, the contents of the plate were allowed to react for exactly 20 min with o-phenylenediamine, the substrate for peroxidase. The plate was measured at 490 nm with a microplate reader after reaction was terminated by addition of 4.5 mol/l sulfuric acid. Mean values per 10^3 cells were determined in triplicate wells for each experiment.

Statistical Analysis

Each assay was repeated at least three times. Statistical comparisons were made using Student t test for unpaired two-group samples. Two-tailed tests were performed, and a p value of < 0.05 was considered significant. Data were expressed as the means ± SD.

Results

Both freshly isolated human fibroblasts (KiF) and the human lung fibroblast cell line (WI-38) exhibited spindle shapes (Fig. 1) and were immunohistochemically α-actin negative (data not shown), which is typical of fibroblast cells. Under the present culture conditions, cells other than fibroblasts, such as alveolar epithelial cells or vascular endothelial cells, were completely eliminated by the actively growing fibroblasts before the 5th passage. The growth rate of WI-38 was significantly higher than that of KiF at 24, 96 and 144 h after seeding (Fig. 1).

Before determination of PAI-1 expression in both human lung fibroblasts, the type of polymorphism in the human PAI-1 promoter gene in both cells was examined. The upstream region of the PAI-1 gene was amplified by PCR, and the products (98 bp) were digested using the restriction

Fig. 1. Growth rates of the two human lung fibroblast strains. Five thousand KiF (●) or WI-38 (■) cells were seeded into 96-well plates containing growth medium and 10% FCS. Cells allowed to attach to the bottom of the plates for 24 h, and cells were counted. Values are means ± SD. Differences between KiF and WI-38 cells were compared for each time point. *: p < 0.05. Photographs of each human lung fibroblast were also shown in the graph. The bar in each photograph at the right bottom indicates 10 μm.
enzyme \textit{Bsl I} \cite{ref39}. The PCR product from WI-38 separated into 72-bp and 22-bp fragments after the digestion with the restriction enzyme (Fig. 2). On the other hand, the amplified PAI-1 gene fragment from KiF remained as a single band after the digestion. This suggests that the PAI-1 promoter sequence in KiF was the deletion (4G) allele and that the sequence in WI-38 was the insertion (5G) allele. To examine the expression of PAI-1 protein, the amounts of protein in cell lysate and conditioned medium (CM) obtained from KiF and WI-38 cells were determined by Western blotting. The PAI-1 band was detected in all cellular and CM samples for both KiF and WI-38 cells, but the KiF cells secreted much larger amounts of PAI-1 into CM than WI-38 did (Fig. 3). The addition of dexamethasone alone had no effect on PAI-1 production (data not shown).

TNF\textsubscript{a} promotes NF-\kappaB binding to the nuclear DNA\cite{ref37}, and dexamethasone inhibits this effect\cite{ref38}. The NF-\kappaB translocation and its inhibition were compared in the nuclear extracts from KiF and WI-38 by the EMSA method (Fig. 4). In the nuclear extract of KiF (4G fibroblasts), dexamethasone was effective within 5 min after addition, but not after 15 min. By contrast, the DNA-bound NF-\kappaB in the nuclear extract of 5G WI-38

Fig. 2. Polymorphism of PAI-1 promoter gene. The promoter region of the human PAI-1 gene in the genomic DNA of KiF (lanes 1, 2) and WI-38 (lanes 3, 4) cells was amplified by PCR (lanes 1, 3). PCR products were digested with the restriction enzyme \textit{Bsl I}, and were electrophoresed on 4.0% agarose gel (lanes 2, 4). Arrows indicate the bands appearing after digestion.

Fig. 3. Expression of PAI-1 protein by human lung fibroblasts. KiF (lanes 1, 2, 3) and WI-38 (lanes 4, 5, 6) cells were pre-incubated with or without TNF\textsubscript{a}. After 4 hours, cells were treated with blank serum-free medium (lanes 1, 4) containing TNF\textsubscript{a} (lanes 2, 5) or TNF\textsubscript{a} and dexamethasone (lanes 3, 6). After 6 h, conditioned medium (CM) was collected and cells were also lysed for examination of PAI-1 by the Western blotting.

Fig. 4. The formation of NF-\kappaB-DNA complex. KiF and WI-38 were treated with or without TNF\textsubscript{a} or dexamethasone as described in the materials and methods section. At 5 and 15 min after the addition of dexamethasone, nuclear extracts were prepared, and the extracts including equal amount of total protein were subjected to electrophoresis. When NF-\kappaB binds specifically to a labeled DNA sequence (arrows), it migrates slower than non-bound DNA using control or cold probe in the electrophoresis gel. The blotted and immobilized bands were reacted with the streptavidin-HRP conjugate and visualized by the reaction of HRP with the chemiluminescence active substrate.
Dexamethasone regulates PAI-1 in lung fibroblasts

**Fig. 5.** Time course of PAI-1 mRNA expression. After both KiF and WI-38 cells were pre-treated with or without TNFα for 4 h, the cells were treated with blank serum-free medium (solid bar) containing TNFα (dashed bar) or TNFα and dexamethasone (open bar) for 0.5, 2, and 8 h. Total RNA was extracted and PAI-1 mRNA was examined by RT-PCR. Photographs of KiF and WI-38 PCR products harvested at 0.5 and 2 h, respectively, are shown in each graph. In each lane, a single band having an identical number of base pairs, 402 and 540, as predicted by the primers specific for PAI-1 and GAPDH mRNA, respectively, was seen. The amounts of PAI-1 mRNA were calculated using Scion image and were normalized against the amount of GAPDH mRNA. Values are means ± SD, and differences were compared for each time point. *: p < 0.05.

**Fig. 6.** Amount of PAI-1 in conditioned medium (CM). KiF and WI-38 cells pre-incubated with or without TNFα were treated with blank serum-free medium (solid bar) containing TNFα (dashed bar) or TNFα and dexamethasone (open bar). After 3, 6 and 9 h, treated CM was collected, and the amounts of PAI-1 were measured by ELISA. Values are means ± SD. Differences in the amounts of PAI-1 antigen were compared for each time point. *: p < 0.05.
was suppressed completely at 15 min after the addition of dexamethasone but not at 5 min.

The amounts of PAI-1 mRNA in KiF and WI-38 cells were examined after the cells were pre-incubated with TNFα and subsequently treated with dexamethasone. In KiF cells, the dexamethasone treatment completely suppressed TNFα-stimulated PAI-1 mRNA expression within 30 min (Fig. 5), although significant differences between TNFα-stimulated and dexamethasone-treated cells were not detected at 2 h. However, after 8 h of dexamethasone treatment, 30% increase in the expression of PAI-1 mRNA was seen over unstimulated cells. On the other hand, the amount of PAI-1 mRNA induced by TNFα was significantly suppressed from 2 to 8 h after addition of dexamethasone in WI-38.

No significant differences in either fibrinolytic activity on the cell surface or the inhibition of secreted PAI-1 between the two fibroblast strains were detected (data not shown). The amount of PAI-1 protein in CM was also measured by the ELISA method. The total amount of secreted PAI-1 from KiF cells was larger than that from WI-38 cells (Fig. 6). In KiF fibroblasts, the addition of dexamethasone suppressed the release of PAI-1 for at least 6 h, but secretion of PAI-1 increased 50% compared with unstimulated cells at 9 h after addition of dexamethasone. Also, the WI-38 cells treated with dexamethasone for 9 h stimulated PAI-1 secretion to double the amount seen in unstimulated cells. Even at 12 h of treatment with dexamethasone, the amounts of secreted PAI-1 from both strains of fibroblasts showed a similar pattern as those at 9 h (data not shown).

Discussion

We showed that two strains of human lung fibroblasts, KiF and WI-38 cells, had distinct types of the PAI-1 promoter gene, the deletion (4G) and insertion (5G) allele, respectively (Fig. 2). The growth rate of KiF was lower than that of WI-38 cells (Fig. 1), but PAI-1 production was markedly higher in KiF cells than in WI-38 cells (Fig. 3). KiF cells mainly secreted PAI-1 into the culture medium, while the corresponding PAI-1 secretion for WI-38 was much smaller. This confirmed a previous report showing that the deletion gene produces PAI-1 at higher levels than the insertion gene.

PAI-1 is the major inhibitor of uPA, and uPA is involved in dissolution of the extracellular matrix and activation of metalloproteinases or growth factors during tissue degradation or stromal remodeling in acute and chronic lung injuries. Pulmonary fibrosis is a disorder of the alveolar wound repair, and characterized by fibroblast proliferation, accumulation of PAI-1 and extracellular matrix remodeling. To maintain normal lung function, tight control of proteolytic enzymes and their inhibitors is needed, and Chuang-Tsai et al. found that PAI-1-deficient mice exhibit reduced formation of fibrotic tissue with bleomycin treatment. Thus, PAI-1 promoter gene polymorphism may be closely related to the progression of pulmonary fibrosis. In the interstitium of injured blood-air barrier, a variety of pro-fibrotic cytokines such as transforming growth factor-β1 and TNFα, are accumulated. PAI-1 expression is regulated by NF-κB. Corticosteroids are routinely used in the treatment of pulmonary fibrosis. One of the corticosteroid mechanisms is inhibition of transcription through NF-κB. Therefore, the effects of dexamethasone on the expression of PAI-1 were compared between KiF (deletion allele) and WI-38 (insertion allele) cells, after stimulation with a pro-fibrotic cytokine, TNFα.

The binding of NF-κB to DNA in 4G KiF was faster than in 5G WI-38 (Fig. 4). In KiF cells pre-treated with TNFα, PAI-1 mRNA suppression was seen only 30 minutes after the addition of dexamethasone, but dexamethasone treatment also gave significant stimulation of PAI-1 mRNA expression after 8 h (Fig. 5). On the other hand, the amount of PAI-1 mRNA was significantly reduced at 2 to 8 h after dexamethasone treatment in WI-38 cells pre-treated with TNFα (Fig. 5). The amount of secreted PAI-1 from TNFα pre-treated KiF was suppressed until 6 hours after the addition of dexamethasone, but increased significantly in both strains of fibroblasts at 9 h after addition (Fig. 6). Because NF-κB regulates the expression of PAI-1 mRNA with TNFα stimulation, dexamethasone would affect NF-κB...
Dexamethasone regulates PAI-1 in lung fibroblasts

translocation or binding to the PAI-1 promoter gene. The binding affinity of NF-κB to the position on the PAI-1 promoter site where the polymorphism occurs may influence the effect of dexamethasone on PAI-1 production. This suggests that PAI-1 production by human lung fibroblasts upon stimulation by TNFα and the effects of dexamethasone closely depend on the PAI-1 promoter gene polymorphism.

The bronchial alveolar fluid of the healthy lung contains uPA antigen to maintain alveolar homeostasis. It is thought that uPA produced and secreted onto the alveolar surface removes substances interrupting the smooth alveolar space. We also reported previously that human lung fibroblasts produce large amounts of PAI-1, but small amounts of uPA. When the inhibition activity in the CM and fibroblast surface uPA activity were examined, there were no statistically significant differences between the fibroblast strains based on the addition of dexamethasone or TNFα (data not shown). The three forms of PAI-1 in the CM are the free active form, the inactive form, and the inactive complex bound with uPA. PAI-1 secreted from lung fibroblasts inactivates uPA by binding to it, or PAI-1-uPA-uPAR complexes on the cell surface are internalized and digested in lysosomes. Although there were large amounts of PAI-1 in the culture medium of 4G KiF, no high molecular weight complex bands were detected in the CM by Western blotting (data not shown). Thus, most of the PAI-1 in KiF CM was in the inactive form. To dissolve the surrounding extracellular matrix, uPA is mainly activated on the cell surface. And uPA, uPAR, and PAI-1 are closely correlated with pulmonary injury and repair through cell migration by activating plasminogen, metalloproteinases or hepatocyte growth factors. PAI-1 also leads to cell detachment by deactivation and internalization of integrins that are bound to uPAR. This function does not require fibrinolytic inhibitory activity, and influences tissue repair or remodeling. The PAI-1 secreted from KiF cells might act as a pulmonary remodeling regulator, even if it is in the inactive form. Because migration of alveolar epithelial cells and vascular endothelial cells into the injured area is very important for pulmonary tissue repair, it seems likely that these cells in the surrounding tissue are influenced by PAI-1 from the fibroblasts. Dexamethasone treatment for more than 9 h might thus affect pulmonary repair, particularly in patients with the 4G allele of the PAI-1 promoter gene.

In summary, dexamethasone reduced the NF-κB translocation and PAI-1 mRNA expression in TNFα-stimulated human lung fibroblasts. This suppression was more rapid in KiF cells, which have the deletion allele of the PAI-1 promoter gene, than in WI-38 cells, which have the insertion allele. However, the expression of PAI-1 mRNA in 4G fibroblasts increased at 8 h after the addition of dexamethasone. The amount of PAI-1 secretion from the fibroblast strains was also higher than in the insertion WI-38 fibroblasts. Secretion was suppressed significantly until 6 h after addition of dexamethasone, but PAI-1 secretion also increased after 9 h. Because longer dexamethasone application appeared to stimulate PAI-1 production and secretion, particularly in KiF having the deletion allele PAI-1 gene, large amounts of secreted PAI-1 from fibroblasts may have induced detachment of surrounding cells, such as vascular endothelial cells or alveolar epithelial cells. Since migration of the alveolar epithelial cells and the vascular endothelial cells into the injured area is important for tissue repair, dexamethasone treatment might suppress pulmonary repair in a patient who has the 4G allele of the PAI-1 promoter gene. Pulmonary tissue-remodeling or fibrotic progress may be predicted by screening for PAI-1 promoter gene polymorphism.

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References

Dexamethasone regulates PAI-1 in lung fibroblasts


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