Hepatocyte growth factor inhibits the formation of the basement membrane of alveolar epithelial cells in vitro

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Furuyama, Akiko, and Katsumi Mochitate. Hepatocyte growth factor inhibits the formation of the basement membrane of alveolar epithelial cells in vitro. Am J Physiol Lung Cell Mol Physiol 286: L939–L946, 2004. First published December 12, 2003; 10.1152/ajplung.00238.2002.—Hepatocyte growth factor (HGF) is a pleiotropic factor for the regeneration of injured pulmonary tissue. We investigated the role of HGF in basement membrane formation during wound healing by immortalized alveolar type II epithelial cells that could form a continuous basement membrane when they were cultured on collagen fibrils in the presence of entactin-contaminated laminin-1. Cells cultured with 5.0 ng/ml HGF neither formed a continuous basement membrane on collagen fibrils nor maintained a continuous basement membrane architecture on a basement membrane substratum. The cells showed increased secretion of matrix metalloproteinase-9 and urokinase-type plasminogen activator, and the HGF-induced inhibition of basement membrane formation was attenuated by addition of 200 ng/ml tissue inhibitor of matrix metalloproteinase-1. Cells sequentially exposed to HGF and 1.0 ng/ml transforming growth factor-β1 had enhanced basement membrane formation compared with those receiving these reagents in the reverse order or concurrently. HGF simultaneously stimulated proliferation and migration of the cells so that it advanced wound closure on the basement membrane substratum. The present results indicate that the role of HGF in wound healing is the stimulation of reepithelialization, but this factor may also contribute to the degradation of the basement membrane.

THE BASEMENT MEMBRANE beneath epithelial and endothelial cells has a highly integrated architecture composed of extracellular matrix (ECM) molecules, including type IV collagen, laminin, entactin (nidogen), and heparan sulfate proteoglycan. Depending on the biological activities of each component, the basement membrane can regulate various kinds of cellular functions such as adhesion, migration, proliferation, and differentiation (1, 4). In the lung, individual basement membrane beneath alveolar epithelial and endothelial cells merges to form a thin layer that is an essential structure of the air-blood barrier. Because alveolar epithelial tissue is always at risk of exposure to external stimuli such as toxic air pollutants and microorganisms, reepithelialization and repair of the basement membrane play an important role in maintaining the barrier function.

The migration and proliferation of type II epithelial cells, their differentiation into type I epithelial cells, and remodeling of the basement membrane are essential processes for repairing alveolar epithelial tissue during wound healing. Cultured type II alveolar epithelial cells cannot form a continuous basement membrane by themselves. Although pulmonary fibroblasts have a key role in producing the acellular interstitium that maintains the integrity of the pulmonary architecture, they also secrete basement membrane constituents sufficient for the assembly of a continuous basement membrane by alveolar epithelial cells (9). Moreover, fibroblasts are a source of cytokines that regulate basement membrane formation (7). Transforming growth factor-β1 (TGF-β1) is one of the cytokines involved in ECM metabolism; this molecule is released from fibroblasts and inflammatory cells (24) during development, inflammation, and tissue remodeling of the lung. Previously, we demonstrated that alveolar epithelial cells cultured on a fibrillar type I collagen substratum supplemented with TGF-β1 at 1.0 ng/ml formed a continuous basement membrane through upregulation of the synthesis of the major basement membrane constituents. Meanwhile, cells treated with TGF-β1 at 5.0 ng/ml increased deposits of cellular fibronectin and type I collagen that prevented the integration of basement membrane constituents beneath the cells (8). Besides TGF-β1, pulmonary fibroblasts secrete various cytokines that modulate ECM production and stimulate lung morphogenesis during development (29). Therefore, we postulated that other cytokines released from fibroblasts and inflammatory cells quite likely participate in the regulation of basement membrane formation by epithelial cells, thereby affecting wound healing.

Hepatocyte growth factor (HGF, also known as scatter factor) is a pleiotropic factor for the regeneration of injured lung (23, 32) and a growth factor for hepatocytes, melanocytes, keratinocytes, and endothelial cells (15). Through its receptor encoded by c-met, HGF exerts multiple biological activities such as the stimulation of epithelial cell proliferation and mobility (28) and the induction of branching tubule formation. In the lung, alveolar macrophages, endothelial cells (30), bronchial cells (33), and fibroblasts (28) produce HGF. HGF dose dependently increases DNA synthesis in adult rat alveolar epithelial cells (14) and induces the proliferation and lumen formation of murine lung epithelial cells cultured on Matrigel (25). Because of the increased secretion in injured lung, HGF is presumed to play an important role in the wound healing of pulmonary epithelium (32). Reepithelialization is a crucial event during early phase of wound healing, whereas basement membrane formation is a necessary process during the late phase. Except for its role in the enhancement of proliferation, little is known regarding the participation of HGF in the formation of
basement membrane architecture. Many of the cytokines, including HGF and TGF-β1, are released after lung injury and are expected to take part in various steps of wound healing. Therefore, it is important to elucidate the physiological roles of HGF in combination with other cytokines such as TGF-β1, which is another key cytokine in the formation of the basement membrane during wound healing.

In the present study, we investigated the effects of HGF in the wound healing process, focusing on the formation and maintenance of the basement membrane by alveolar epithelial cells in vitro. Our results revealed that although it stimulated the proliferation and migration of alveolar epithelial cells, HGF inhibited formation of the basement membrane. Temporal expression of HGF soon after injury contributed to wound healing by reepithelialization. Exposure to HGF followed by replacement of HGF by TGF-β1 at the late phase was most efficacious for basement membrane formation of alveolar epithelial cells in vitro.

MATERIALS AND METHODS

Reagents. Recombinant human HGF and human platelet-derived purified TGF-β1 were purchased from R&D Systems (Minneapolis, MN). Entactin-contaminated laminin-1 (LN/EN), Matrigel, and rabbit anti-laminin-1 antibody were purchased from BD Bioscience (Bedford, MA). Recombinant human tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) was purchased from Fuji Chemical Industry (Takaoka, Japan). Rabbit anti-type IV collagen antibody was purchased from Progen Biotechnik (Heidelberg, Germany). Goat anti-matrix metalloproteinase-2 (MMP-2) antibody was purchased from Oncogene Research Products (Boston, MA). Rabbit anti-MMP-9 antibody, rabbit anti-TIMP-1 antibody, and rabbit anti-TIMP-2 antibody were purchased from Chemicon International (Temecula, CA). Rabbit anti-plasminogen activator inhibitor-1 (PAI-1) antibody and rabbit anti-u-urokinase-type plasminogen activator (uPA) antibody were purchased from American Diagnostica (Greenwich, CT).

Cell culture. Immortalized alveolar type II epithelial cells (obtained from rats transfected with SV40-large T antigen gene; SV40-T2 cells) were a gift from Dr. A. Clement (Hôpital Armand Trousseau, Paris, France) (3). They were maintained in DMEM supplemented with 10% FBS and 0.2 mM ascorbic acid 2-phosphate and examined with a microscope (Wako Pure Chemical Industries). The effect of HGF on cell migration was evaluated by a scratch wound assay. SV40-T2 cells at a density of 1.0 × 10^5 cells/well were cultured on the basement membrane substratum for 3 days, and then the cell monolayer was wounded with the edge of a plastic pipette tip with a width of ~0.3 mm. The wounded tissues were cultured in the presence or absence of 5.0 ng/ml HGF with or without 200 ng/ml TIMP-1 in DMEM with 1% FBS and 0.2 mM ascorbic acid 2-phosphate and examined with a Nikon diaphot microscope (Nikon, Tokyo, Japan) at 0, 8, and 24 h after wounding. To stop the cell proliferation, we added 5 mM thymidine to the medium 24 h before scratching. The rate of reepithelialization was expressed as the restoring width, that is, [(the initial wound area – the exposed area at 8 h/the wound length). For quantification of the wound closure, the exposed basement membrane substratum was manually outlined with the Lasso tool in the toolbox of Photoshop. Using the Histogram tool in the image menu, we obtained the wound width as the number of pixels on exposed area vs. the number of pixels on the wound length × 1 μm. For quantification of cell spreading, the Magic Wand tool was adjusted to one dark nucleus. We automatically selected and counted entire nuclei using the Similar command in the select menu. For quantification of the basement membrane formation, three representative fields (magnification ×40) were chosen in the immunostained tissues. The Magic Wand tool was used to select the bright-stained basement membrane-containing architecture. The pixel number on the bright-stained area was generated with the Histogram tool. The quotient (total number of white pixels/total pixels examined) was a reflection of deposit area (%).

Gelatin, casein, and casein-plasminogen zymography. SV40-T2 cells were cultured on collagen fibrils in serum-free medium were treated with 5.0 ng/ml HGF for 24 h. The cultured media were concentrated with 5.0 ng/ml HGF for 24 h. The cultured media were concentrated 10-fold with filtrate unit (Millipore, Bedford, MA) and suspended in diithiothreitol-deficient sample buffer and analyzed for gelatinolytic activity. Samples controlled for cell number were run on 10% SDS-PAGE containing 0.5 mg/ml porcine skin gelatin (Sigma, St. Louis, MO) under nonreducing conditions. To evaluate plasminogen activator activities, samples were also run on 10% SDS-PAGE containing 1.0 mg/ml casein (Sigma) with or without 13 μg/ml plasminogen (Technoclone, Vienna, Austria). The gels were washed in 2.5% Triton X-100 and then incubated for 24 h at 37°C in incubation buffer of 50 mM Tris (pH 7.6) with 150 mM NaCl, 10 mM CaCl_2, and 0.02% NaN_3. The gels were stained with Coomassie brilliant blue R250 and destained with 50% methanol and 7.5% acetic acid. Pro-MMP-2 and pro-MMP-9 (Wako Pure Chemicals) were run as markers.

Western blot analysis. The effect of HGF on the secretion of ECM and various enzymes from SV40-T2 cells was assessed by Western blot analysis. SV40-T2 cells cultured on collagen fibrils in serum-free medium were treated with 10.0 ng/ml HGF for 4 days. The conditioned medium from laminin-1 and type IV collagen, 200 ng/ml TIMP-1 was added to the media. Cultured media supplemented with 5 μg/ml leupeptin, 5 μg/ml pepstatin A, 1 μg/ml antipain, 1 μg/ml chymostatin, 5 μg/ml phospho...
phoramidon, and 2 mM phenylmethylsulfonyl fluoride were mixed with bovine serum albumin at a final concentration of 0.5 μg/ml. Trichloroacetic acid was added to the media at a final concentration of 0.1 ml/ml and kept in ice for 30 min. These media were centrifuged at 3,500 rpm for 20 min, and precipitates were dissolved in SDS-PAGE sample buffer to be concentrated 30-fold. Samples controlled for cell number were separated by 10 or 5% polyacrylamide gel under reducing conditions. For uPA detection, precipitated media were suspended in dithiothreitol-deficient sample buffer and were separated by 10% polyacrylamide gel under nonreducing conditions. The bands were electrophoretically transferred to a polyvinylidene fluoride membrane (Millipore). Proteins were immunologically identified with primary antibodies for MMP-2, uPA, MMP-9, TIMP-1, TIMP-2, PAI-1, laminin-1, and type IV collagen and matched peroxidase-conjugated antibodies (Sigma). The immunoreactive bands were revealed and recorded by ECL Plus Western blotting detection kits and chemiluminescence films (Amersham International, Buckinghamshire, UK).

**RESULTS**

HGF inhibited basement membrane formation by cultured alveolar epithelial cells. SV40-T2 cells cultured on a fibrillar type I collagen substratum cannot form a continuous basement membrane (7–9). TEM revealed immature lamina densa-like fragments of discontinuous electron-dense deposits beneath the cells (Fig. A1A), and the addition of 5.0 ng/ml HGF reduced the size and number of these deposits (Fig. 1B). The cells were able to form a completely continuous lamina densa when they were cultured on the fibrillar type I collagen supplemented with 50 μg/ml LN/EN (Fig. 1C). However, in the presence of 5.0 ng/ml HGF, the cells failed to form a continuous lamina densa even when cultured with LN/EN (Fig. 1D). The formation of basement membrane by the cells was dose dependently inhibited by HGF at concentrations of 0.2, 2.0, 5.0, and 10 ng/ml (data not shown).

The assembly of the major constituents of the basement membrane as evaluated by confocal laser scanning microscopy corresponded with the formation of the lamina densa as monitored by TEM. The cultured tissues were stained for laminin-1 (Fig. 2) or type IV collagen (data not shown). Horizontal views beneath the SV40-T2 cells in the absence of LN/EN revealed the meshwork of an immature basement membrane-like structure (Fig. 2A). In the presence of 5.0 ng/ml HGF, openings of the meshwork beneath cells were more extensive (Fig. 2B). Moreover, the formation of a continuous sheet of basement membrane beneath cells that were cultured with LN/EN (Fig. 2C) was inhibited in the presence of 5.0 ng/ml HGF (Fig. 2D). These results indicate that HGF prevents SV40-T2 cells from forming a basement membrane.

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Fig. 1. Transmission electron micrographs beneath rat alveolar epithelial cells cultured in the presence of hepatocyte growth factor (HGF). SV40-T2 cells (A–D) were cultured for 10 days on fibrillar type I collagen without (A and C) or with (B and D) 5.0 ng/ml HGF in the absence (A and B) or presence (C and D) of 50 μg/ml enactin-contaminated laminin-1 (LN/EN). Electron-dense deposits (arrows) were observed beneath cells both without (A) and with (B) HGF, and the deposits beneath the cells cultured with HGF were reduced. Cells that were cultured in the presence of LN/EN could form a continuous lamina densa (C). However, the lamina densa was broken (arrowheads) beneath cells cultured with HGF even in the presence of LN/EN (D). SV40-T2 cells were cultured on fibrillar type I collagen with 5.0 ng/ml HGF in the presence of 200 ng/ml tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) and 50 μg/ml LN/EN for 10 days. A continuous lamina densa (arrows) was observed beneath cells (E). Bar, 200 nm. All micrographs are representative of the particular experimental group.

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HGF promoted disassembly of the basement membrane architecture. We devised a method for culturing SV40-T2 cells on a basement membrane substratum to assess whether exposure to HGF promoted degradation of the basement membrane architecture. The basement membrane substratum was prepared from a tissue of SV40-T2 cells cultured in the presence of 100 µl of Matrigel as described previously. The substratum consisted of a continuous lamina densa on fibrillar type I collagen and the lamina densa preserved continuous deposits of the major basement membrane constituents (data not shown). In the absence of HGF, SV40-T2 cells adhered to the basement membrane substratum, formed anchoring filaments between the basal cell surface and the lamina densa, and maintained the continuous architecture of the basement membrane for 10 days (Fig. 3B). However, in the presence of 5.0 ng/ml HGF, the continuous lamina densa became nicked, and small gaps were observed (Fig. 3C). In the presence of 50 µg/ml LN/EN, HGF-induced nicks on the lamina densa were reduced. These results suggest that HGF-treated SV40-T2 cells promote the disassembly of the continuous architecture of the lamina densa.

HGF induced gelatinolytic and plasminogen activator activities of alveolar epithelial cells. To explore which factors might be associated with HGF-induced inhibition of basement membrane formation, we analyzed the gelatinolytic activity of alveolar epithelial cells by gelatin zymography. Basal cell membrane formation is an accumulative process of basement membrane constituents, and the balance between synthesis and degradation during initial stages affects the fate of the basement membrane assembly. We measured the secretion of uPA and various MMPs at days 3, 5, and 8 of culture; we show the results of day 3 as representative data (Fig. 4). Although SV40-T2 cells in the absence of HGF lacked MMP-9 activity, treatment with 5.0 ng/ml HGF weakly induced the secretion of pro-MMP-9 activity in the culture medium (Fig. 4A). Western
blot analysis confirmed the induction of pro- and active-MMP-9 (Fig. 4B). The activity of MMP-2 was very low and was unchanged by HGF treatment. The activity of plasminogen activator was revealed by casein-plasminogen zymography. Whereas SV40-T2 cells cultured in the absence of HGF had no uPA activity, a plasminogen-dependent proteolytic band corresponding to the inactive single-chain proenzyme form occurred in the culture medium in the presence of HGF (Fig. 4C). Western blot analysis also detected pro- and active forms of uPA in the culture medium of cells treated with HGF (Fig. 4D). Treatment with 5.0 ng/ml HGF did not diminish secretion of endogenous protease inhibitors such as TIMP-1, TIMP-2, and PAI-1 (Fig. 5). HGF had no effect on the production of basement membrane constituents such as laminin-1 and type IV collagen (Fig. 5), and no significant change in the bands corresponding to their degradation was appreciated (data not shown). Molecules were secreted to the medium mainly from the basal side (data not shown). These results indicate that the balance in the basement membrane formation by alveolar epithelial cells shifts from assembly to degradation after treatment with HGF.

Treatment with TIMP-1 abrogated the HGF-associated inhibition of basement membrane formation. SV40-T2 cells cultured with 200 ng/ml TIMP-1 and 50 μg/ml LN/EN could form a continuous basement membrane even in the presence of 5.0 ng/ml HGF (Fig. 1E). Together, these data indicate that the induction and secretion of MMP-9 likely contribute to the HGF-associated inhibition of basement membrane formation.

HGF accelerated reepithelization of tissues of alveolar epithelial cells. To assess the contribution of HGF in combination with TGF-β1 to each step in the wound-healing process in the lung, we evaluated the effects of these factors on the migration, proliferation, and basement membrane formation of SV40-T2 cells. Wound closure assays demonstrate that HGF stimulated the migration of SV40-T2 cells on the basement membrane substratum independent of treatment with thymidine to synchronize the cells at the S phase (Fig. 6A). HGF treatment induced no significant difference in cell number between control and HGF-treated cells by 8 h of culture. Both control and HGF-treated cells in areas adjacent to the wound edge spread well compared with the cells more distant (Fig. 6B). To clarify whether MMPs contributed in the HGF-induced stimulation of migration, wounded tissues were cultured with 5.0 ng/ml HGF in the presence of 200 ng/ml TIMP-1. TIMP-1 had no effect on wound closure by the cells (Fig. 6C).

HGF at 1.0 and 5.0 ng/ml stimulated the proliferation of SV40-T2 cells on a fibroblast type I collagen substratum during a 5-day culture (Fig. 7). In the absence of HGF, the cells failed to reach full confluence, whereas after supplementation with 5.0 ng/ml HGF, they covered the surface of the substratum (data not shown). HGF at 1.0 and 5.0 ng/ml also enhanced the proliferation of SV40-T2 cells on the basement membrane substratum (data not shown). These data indicate that HGF accelerates the reepithelization of SV40-T2 cells by stimulating both cell proliferation and cell migration.

As TGF-β1 is another cytokine expressed during the processes of wound healing, the physiological role of HGF in the basement membrane formation is more precisely elucidated in its relationship to TGF-β1. As shown in our previous report (7), SV40-T2 cells cultured with 1.0 ng/ml TGF-β1 succeeded in forming a continuous basement membrane. When cell density was reduced one-fifth of that of previously, SV40-T2 cells failed to form a complete basement membrane even when cultured with TGF-β1, presumably because of the suppression of proliferation by TGF-β1 (Fig. 8). TGF-β1 at 1.0 ng/ml
suppressed the growth of SV40-T2 cells (Fig. 7). SV40-T2 cells sequentially exposed to 5.0 ng/ml HGF for 5 days and no HGF for the next 5 days showed more basement membrane formation than untreated cells (Fig. 8). Therefore, the HGF-associated increase of cell number during early phases supported basement membrane formation, and the HGF-induced degradation of basement membrane did not continue after removal of HGF. Furthermore, SV40-T2 cells sequentially exposed to 5.0 ng/ml HGF for 5 days and 1.0 ng/ml TGF-β1 for the next 5 days showed enhanced formation of basement membrane compared with both untreated cells and those exposed to either cytokine for 10 days. Cells simultaneously exposed to HGF and TGF-β1 or exposed to TGF-β1 then HGF deposited fewer laminin-1 than did the cells treated in the reverse order. Therefore, HGF in the late phases abrogated the stimulatory effects of TGF-β1 on basement membrane formation. These results indicate that the temporal expression of HGF soon after injury followed by replacement of HGF with TGF-β1 at late phases supports for basement membrane formation.

DISCUSSION

We demonstrated that HGF perturbs the formation of the basement membrane by alveolar epithelial cells. HGF diminished the deposits of both endogenous and exogenous basement membrane constituents. Once the basement membrane architecture had been assembled, however, HGF-associated degradation was slight. Although HGF stimulates expression of the laminin γ2-chain gene in human intestinal epithelial cells (22), HGF failed to enhance the secretion of laminin-1 or the α5-chain of type IV collagen in rat alveolar epithelial cells. Moreover, HGF induced both the proteolytic activities and protein levels of MMP-9 and uPA but not the production of TIMP-1, TIMP-2, or PAI-1. In response to HGF, the balance between the synthesis and degradation of basement membrane constituents was shifted toward degradation of the constituents. HGF-induced inhibition of basement membrane formation was blocked by supplementation with 200 ng/ml TIMP-1. Therefore, we attribute the reduced basement membrane formation by HGF-treated SV40-T2 cells at least in part to HGF-associated induction of MMP-9.

The basement membrane serves as a scaffold for the adhesion of epithelial cells, and various adhesion molecules mediate this adhesion (1, 4). MMPs, which are zinc-dependent metalloendoproteinases (19, 20), are thought to regulate cellular behavior by their influence on the interaction between adhesion and ECM molecules. MMP-2 and MMP-9 can degrade gelatin, type IV collagen, type V collagen, fibronectin, laminin, and type III collagen (19, 20). Normal rat alveolar type II cells produce MMP-2, and the treatment with lipopolysaccharide (LPS) or interleukin-1 increased the production of MMP-2 and MMP-9 in vitro (5). Western blot analysis did not reveal MMP-1 in the culture medium of HGF-treated SV40-T2 cells (data not shown), although HGF stimulates the production of MMP-1 (7) and MMP-9 (16) in keratinocytes. The secretion of pro-MMP-9 from SV40-T2 cells was induced by HGF treatment, and they produced only trace amounts of MMP-2. The MMP-9 released from the basolateral surface of SV40-T2 cells was efficient for degrading the basement membrane constituents nearby. The fact that the lamina densa architecture beneath the HGF-treated cells showed only small gaps suggests that the assembled basement membrane constituents are more resistant to MMPs than are the free macromolecules.

Plasminogen activators are serine proteases responsible for the conversion of inactive plasminogen into active plasmin.
Rat pulmonary alveolar epithelial cells produce uPA, and cellular differentiation and inflammatory mediators such as LPS and tumor necrosis factor influence its expression (10). HGF induces uPA in both normal and tumor cells (6). In turn, plasmin can degrade ECM components such as lamini, fibronectin (13), and proteoglycans (17) during cell migration and tissue remodeling. Plasmin is an endogenous factor for the proteolytic activation of pro-MMPs (12, 27). In addition, uPA can convert the HGF precursor protein to the active form (18). We used recombinant human HGF, which was a mixture of the immature single prepropeptide chain and the mature disulfide-linked heterodimeric glycoprotein comprising α- and β-subunits. Therefore, the immature single chain of HGF may become active owing to not only proteases in FBS but also the induced uPA of SV40-T2 cells treated by HGF all the more. Our present results indicate that HGF-mediated simultaneous induction of MMP-9 and uPA activity in alveolar epithelial cells synergistically inhibits basement membrane formation. Although several studies have shown that HGF upregulates various proteases (6, 16), the transcriptional regulatory mechanisms for the induction of MMPs are still unclear. The HGF receptor is a transmembrane tyrosine kinase (2). HGF enhances tyrosine phosphorylation of β-catenin and suppresses the function of the cadherin-mediated cell-cell adhesion system (26). The HGF-induced scattering of SV40-T2 cells probably reflects this perturbation of tightly bound cell-cell adhesion. Furthermore, disruption of the cell-cell adhesion likely induces cytoskeletal reorganization, which in turn activates the extracellular signal-regulated kinase signaling pathway to mediate uPA expression (11). In this way, signaling through the HGF receptor probably influences the gene expression induced by cytoskeletal reorganization and subsequent signal transduction pathways. These correlations suggest that HGF-mediated induction of synthesis of MMPs and uPA, cell proliferation, and migration may be interrelated during normal wound healing.

Cell proliferation and migration are crucial processes in wound healing. At an early step in wound healing, alveolar epithelial cells must promptly cover the denuded surfaces of the basement membrane and interstitial ECM of alveolar septa. The formation and repair of the basement membrane are the next steps in the restoration of the air-blood barrier structure. In our study, HGF stimulated the proliferation and migration of SV40-T2 cells on the basement membrane substratum in a dose-dependent manner. These data support the hypothesis that exposure to HGF soon after injury facilitates reepithelialization of damaged lung tissue by alveolar epithelial cells. The cell migration induced by HGF was not affected by TIMP-1 in wound closure assays, in contrast to the neutralization of HGF-induced inhibition of basement membrane formation by TIMP-1. Although HGF-treated cells produced pro-MMP-9 after 3 days, induction of pro-MMP-9 was not confirmed after 8 h (data not shown). Therefore HGF-associated induction of MMP-9 may not be essential to promotion of the migration of SV40-T2 cells. Various cytokines released after lung injury take part in each phase of wound healing. In the lung, mRNA transcription and activity of HGF increase 3–12 h after injury and decline by 24 h (32); these findings indicate that HGF affects alveolar epithelial cells only during the early phase of restoration. TGF-β1 is a key cytokine that enhances ECM production, and the expression of this protein increases during the inflammatory and reparative phases in wound healing (24, 31). Furthermore, HGF and TGF-β1 negatively regulate each other’s expression. The HGF gene has a TGF-β1-responsive inhibitory element in its promoter (21). In turn HGF suppresses the expression of TGF-β1 (34). Furthermore, administration of HGF represses bleomycin-induced fibrotic changes in the murine lung (31). To gain insight into the physiological contribution of HGF in combination with TGF-β1 during wound healing in vivo, we examined the formation of the basement membrane by alveolar epithelial cells treated with both HGF and TGF-β1 in vitro. HGF diminished TGF-β1-stimulated basement membrane formation (Fig. 8). However, HGF-associated induction of cell proliferation was effective for the basement membrane formation, as long as the factor’s expression was limited to the early phase. Cultured alveolar epithelial cells treated with HGF and TGF-β1 in the same order as occurs in vivo succeeded in representing the normal tissue remodeling of alveoli. The balance between HGF and TGF-β1 may play an important role in the regulation of ECM metabolism and tissue remodeling. Mechanisms for systematic increases in HGF and TGF-β1 and their sequential replacement will be necessary for efficient reepithelization and wound healing in alveolar epithelial tissue.

In conclusion, although HGF ultimately inhibits the formation of the basement membrane by cultured alveolar epithelial cells, the prompt increase of HGF activity soon after lung damage enhances the proliferation and migration of the cells, thereby contributing to efficient reepithelization. Furthermore, we suggest that both HGF and TGF-β1 play important roles in the various aspects of wound healing, including reepithelialization and restoration of the basement membrane.

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REFERENCES


