Basic fibroblast growth factor stimulates repair of wounded hepatocyte monolayer: Modulatory role of protein kinase A and extracellular matrix

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The two important repair mechanisms after hepatocyte injury are proliferation and migration of the nearby healthy hepatocytes. Although previous studies have shown that basic fibroblast growth factor (bFGF) levels are markedly elevated after liver injury, the role of bFGF in the repair of the wounded hepatocytes is not well understood. The aim of this study was to delineate the role of bFGF in the repair of the wounded hepatocyte monolayers. Specifically, we examined the role of bFGF in cellular proliferation and migration of hepatocytes with an in vitro wound model. Standardized excisional wounds were created in clone 9 rat hepatocyte monolayers by a razor blade, and the extent of epithelial proliferation and migration was measured. After wound formation, bFGF (30 ng/mL) significantly stimulated proliferation of hepatocytes at the wound margin. bFGF also stimulated the migration of hepatocytes at the wound front. bFGF stimulation of hepatocyte migration correlated with increased formation of actin stress fibers and bFGF-receptor protein level. The bFGF stimulation of hepatocyte migration was abolished by various protein kinase A activating agents including 3-isobutyl-1-methylxanthine, 8-bromoadenosine-3′, 5′-cyclic monophosphate, forskolin, and cholera toxin. In addition, protein kinase A activating agents almost completely prevented bFGF-induced actin stress fiber formation in the cells at the wound front. Varying the basement membrane composition of the extracellular matrix had a selective enhancing effect on the basal rates of hepatocyte migration (collagen IV ≥ laminin > collagen I > fibronectin > control (plastic)). bFGF treatment resulted in a similar additive increase in hepatocyte migration across all coated surfaces studied. We conclude that bFGF promotes hepatocyte wound repair by stimulating both proliferation and migration of the hepatocyte at the margin of the wound. (J Lab Clin Med 1999;134:363-71)

Abbreviations: BFGF = basic fibroblast growth factor; BrdU = bromodexyuridine; ECM = extracellular matrix; 8Br-cAMP = 8-bromoadenosine–3′,5′-cyclic monophosphate; IBMX = 3-isobutyl-1-methylxanthine; MMC = mitomycin C; PBS = phosphate buffer saline solution; PKA = protein kinase A

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The two important repair processes after wounds to the liver are cellular proliferation and migration. Hepatocyte proliferation is important for the replacement of the apoptotic or necrotic hepatocytes, whereas migration is necessary for the restitution and reconstruction of the liver structure. Basic fibroblast growth factor (bFGF) levels are markedly elevated following liver injury.[1] Although bFGF has been shown

An editorial relevant to this article appears on p. 331 of this issue of the Journal.

to be a potent mitogen and to stimulate intestinal epithelial migration, its role in hepatocyte wound healing is unclear.[2] In this study the role of bFGF in the repair of hepatocyte monolayer wounds was determined with an in vitro wound model.

Previous studies have demonstrated that several growth factors such as transforming growth factor-α, hepatocyte growth factor, and epidermal growth factor play a role in liver restitution.[3-9] Following liver injury, hepatocytes secrete growth factors and cytokines that activate cells adjacent to and distant from the injury site. Secreted growth factors bind to their specific receptors and trigger intracellular processes that could result in hepatocyte proliferation and migration.

Understanding the cellular processes involved in the healing of liver epithelial wounds is important in developing strategies to accelerate the repair of the injured liver. In this study we examined the possible involvement of bFGF in the repair of hepatocyte monolayer wounds following excisional wound formation. Specifically, we tested the hypothesis that bFGF stimulates hepatocyte proliferation and promotes hepatocyte migration following epithelial wound formation. In addition, the possible intracellular mechanisms of bFGF action on hepatocyte migration were investigated.

METHODS

Materials. The culture ware supplies were purchased from Corning Costa Corporation (Cambridge, MA). F12K Kaighn's modification and human recombinant bFGF were purchased from Life Technologies, Inc (Gaithersburg, MD). Fetal bovine serum was purchased from Geminin Bioproducts, Inc (Calabassas, CA). Mayer's hematoxylin-eosin staining solution, trypsin-ethylenediamine tetraacetic acid, 10 mmol/L phosphate-buffered saline solution (PBS) pH 7.4, cholera toxin, 3-isobutyl-1-methylxanthine (IBMX), and 8-bromoadenosin-3′, 5′-cyclic monophosphate (8Br-cAMP) were purchased from Sigma Chemical Co (St Louis, MO). Antibiotics (10,000 U/mL penicillin G, 25 μg/mL amphotericin B, and 10,000 μg/mL streptomycin) were purchased from Cellgro (Washington, DC). Collagen type I, collagen type IV, fibronectin, and laminin were purchased from Collaborative Biomedical Products (Lincoln Park, NJ). Fluorescein-labeled phalloidin was purchased from Molecular Probes, Inc (Eugene, OR).

Cell culture. Liver epithelial cell line (clone 9), originally passaged from normal rat liver, was obtained from American Type Culture Collection (Rockville, MD) at passage 16.[10-14] The stock culture was grown in a culture medium composed of F12K Kaighn's Modification with 1260 mg/mL glucose, 292 mg/mL glutamine, 1000 U/mL penicillin G, 2.5 μg/mL amphotericin B, 1000 μg/mL streptomycin, and 10% fetal bovine serum. Culture medium was changed every 2 days. The cells were subcultured by partial digestion with 0.5 g/L trypsin and 0.2 g/L ethylenediamine tetraacetic acid in Hank's balanced salt solution. The clone 9 hepatocytes were detached from stock cultures by trypsin digestion and subcultured in 20 mL medium in cultured flasks at a concentration of 5×10^5 cells/mL. Cultures were examined on a regular basis under an inverted light microscope to monitor growth and to rule out contamination.

Assessment of cell proliferation. Cell proliferation (DNA synthesis) was determined by measuring the bromodeoxyuridine (BrdU) uptake by the clone 9 cells. Confluent clone 9 monolayers grown on collagen I-coated glass cover slips were pretreated with varying concentrations (0, 1, 2, 3, 4, 5 μg/mL) of mitomycin C (MMC) at 37°C for 2 hours. Standardized excisional wounds, 25 mm wide, were made with a single-edge razor blade. Then clone 9 hepatocyte monolayers were incubated in culture media at 37°C for 24 hours. One hour before the end of the incubation period, 1.0×10^{-4} mol/L BrdU was added to the culture. One hour later monolayers were fixed in a 3.75% formaldehyde solution in PBS for 20 minutes, washed with PBS, and permeabilized in acetone at –20°C for 5 minutes. Hepatocyte monolayers were immunostained as described previously with anti-bromodeoxyuridine antibody (Dakopatts, Glostrup, Denmark).[15] Labeling index, expressed as a percentage of BrdU-labeled cells, was determined by counting a total of 1000 cells at the wound margin with light microscopy. This procedure was done by 2 investigators evaluating coded slides. The rat hepatocyte BrdU uptake was reduced to basal levels at MMC concentrations ≥3 μg/mL.

The [^{3}H] thymidine uptake studies were performed with standard methods as previously described.[2] For [^{3}H] thymidine uptake studies clone 9 hepatocytes were grown in 24-well plates to approximately 50% to 60% confluence. The cells were then incubated in F12K buffer overnight without fetal bovine serum. The hepatocytes were treated with appropriate experimental reagents and incubated in F12K buffer for 24 hours. [^{3}H] thymidine (2.0 μCi/well) was added to each well for the last 3 hours of incubation. At the end of the 24-hour experimental period, incubation solution was removed, and the hepatocytes were rinsed with ice-cold PBS and lysed with 1 N NaOH. The hepatocyte [^{3}H] thymidine uptake was then determined by measuring radioactivity with a liquid scintillation counter.

Migration studies. The newly plated clone 9 hepatocytes were grown to confluence (4 days after plating) on 6-well plates. The confluent monolayers were rinsed with F12K buffer solution, and standardized excisional wounds (25 mm) were made by scraping with a single-edge razor blade as previously described.[16,17] In all experiments hepatocyte monolayers were pretreated with 4 μg/mL MMC for 2 hours before wound formation to prevent cellular proliferation.[18] After the scrape was performed, the "wounded" monolayers were washed twice with F12K buffer solution. Subsequently, appropriate experimental reagents were added, and the wounded monolayers were incubated in F12K buffer solution at 37°C for 24 hours. After the end of the 24-hour incubation period (migration time), the incubation solution was removed, and the cells were fixed in methanol for 5 minutes and stained

with Mayer's hematoxylin-eosin staining solution. Hepatocyte migration was then measured with a computerized image analyzer system composed of a binocular microscope (TMS-F No. 2 Nikon, Tokyo, Japan) attached to a Macintosh computer with the National Institutes of Health Image Program Version 1.58 (National Institutes of Health). The total area of migration across a 1-cm vertical excisional cut was measured with the National Institutes of Health Image Program. For the purpose of analysis, all migration data were standardized to hepatocyte migration (mm^2) across 1 mm of vertical wound. The plates were coded, and measurements were done by a person unaware of the code and not involved in the experiments. For each experiment 3 separate wells (n = 3) were scraped and measured. To ensure reproducibility all experiments were repeated 3 to 6 times.

Coating of extracellular matrix components. Each well of 6-well plastic plates was coated with selected components of extracellular matrix (ECM) including collagen type I, collagen type IV, laminin, or fibronectin. The individual wells were coated by incubation in specific ECM component solution (2 $\mu g/cm^2$) according to the manufacturer's instructions. Wells were subsequently rinsed with either Hank's balanced salt solution or F12K buffer solution. Hepatocytes were then grown to confluence on the ECM-coated wells, and migration experiments were performed as described previously.

Immunofluorescent labeling of actin stress fibers. Clone 9 f-actin filaments were fluorescein-labeled as previously described.[19] Hepatocytes were grown to confluence on glass coverslips coated with collagen I. Subsequently, excisional wounds were made, and the wounded cells were treated with appropriate reagents and incubated for 24 hours. The hepatocyte monolayers were then fixed in a 3.75% formaldehyde solution in PBS. Hepatocyte monolayers were permeabilized in acetone at –20°C for 5 minutes and washed with PBS. Then 5 μU of fluorescein-labeled phalloidin dissolved in 100 mL PBS was placed on each coverslip for 40 minutes. After a PBS rinse was performed, coverslips were mounted on a slide with the cell side down in a 1:1 solution of PBS and glycerol. The distribution of actin microfilaments was examined with a Nikon epifluorescence microscope.

Western blot analysis of bFGF receptor-1 protein. Western blot analysis of bFGF receptor-1 protein was performed with standard methods.[20] After appropriate experimental treatment was performed, clone 9 hepatocyte monolayers were lysed in lysis buffer (50 mmol/L Tris HC1, 5 mmol/L ethylenediamine tetraacetic acid, 10 mmol/L ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid, 0.3% wt/vol b-mercaptoethanol, 10 mmol/L benzamidine, and 50 mg/mL phenylmethylsulphonyl fluoride) and scraped, and lysates were placed in microfuge tubes. The cleared lysates were collected by separation in microfuge. Subsequently, bFGF receptor-1 protein was immunoprecipitated by rabbit anti-bFGF receptor-1 antibody (Sigma Chemical Co). The immunoprecipitated samples containing antigen-antibody complex were then collected and dissolved in SDS gel loading buffer and separated on 7.5% SDS-PAGE gel. The protein from the gel was then transferred to the nitrocellulose filter, and the Western blot analysis was performed with the Western blot kit from the Amersham Corp (Arlington Heights, IL). Densitometry measurements of the individual protein bands were obtained and expressed as pixels.

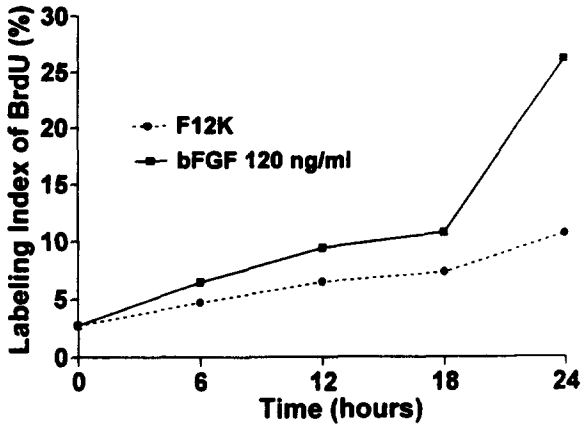

Fig 1. Effect of bFGF on BrdU uptake by hepatocytes at wound front. Confluent clone 9 rat hepatocyte monolayers were wounded by scraping with single-edge razor blade as described in Material and Methods section. After standardized wound formed, BrdU uptake was measured as described in Material and Methods section. Total of 1000 hepatocytes was counted at each time point from 4 separate experiments. Marked increase occurred in BrdU uptake by bFGF (30 ng/mL)-treated hepatocytes at 24-hour time point.

Statistical analysis. Results are expressed as mean ± SEM. Statistical significance of differences between mean values was assessed with Student's *t* tests for unpaired data. All reported significance levels represent 2-tailed *P* values. A *P* value <.05 was used to indicate statistical significance.

RESULTS

Effect of bFGF on hepatocyte proliferation and migration. To assess the effect of bFGF on hepatocyte monolayer wound repair, the effect of bFGF on proliferation and migration of the clone 9 hepatocytes at the wound margin was determined. The effect of bFGF on proliferation of the cells at the wound margin was measured by BrdU labeling (Fig 1). The bFGF treatment (30 ng/mL) resulted in a significant increase in BrdU uptake by the hepatocytes at the wound front at 24 hours, indicating stimulation of hepatocyte proliferation. At earlier time points (6, 12, and 18 hours) BrdU uptake was also increased, but to a lesser degree.

Because reconstitution or re-epithelialization of the wounded hepatocyte monolayer could result from either (or both) cell proliferation or migration of the cells at the wound margin, in the following studies epithelial monolayers were pretreated with MMC for 2 hours before wound formation to ensure that the observed wound re-epithelialization was from cell migration and not proliferation. MMC inhibits DNA synthesis by cross–linking DNA at guanine and adenine residues, and MMC is widely used in the epithelial migration

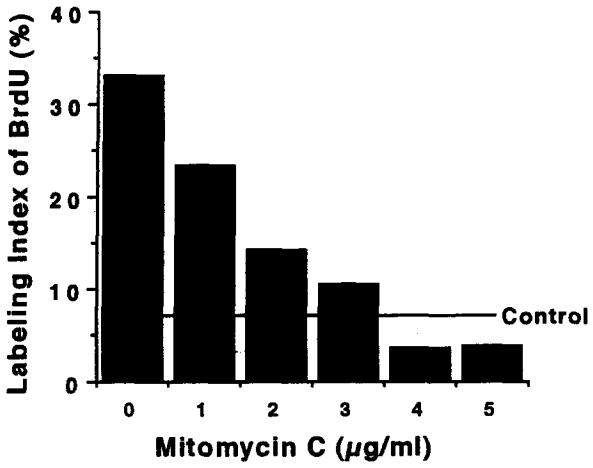

Fig 2. Effect of mitomycin C on rat hepatocyte BrdU uptake. MMC pretreatment for 2 hours inhibited bFGF (30 ng/mL)-stimulated hepatocyte BrdU uptake in dose-dependent manner. Percent BrdU uptake was assessed by counting total of 1000 cells from 3 separate experiments. There was maximal inhibition of bFGF-stimulated BrdU uptake at MMC concentrations ≥4 μg/mL.

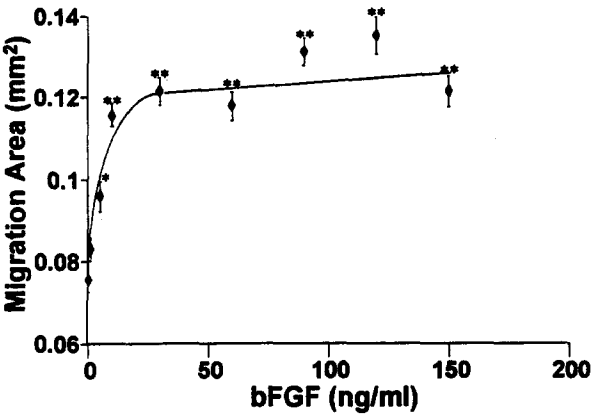

Fig 3. Effect of varying concentrations of bFGF on hepatocyte migration (mm^2). Following wound formation, bFGF was added to incubation solution, and cell migration was measured 24 hours later as described in Material and Methods (n = 4). Data are representative of results obtained from 4 separate experiments.

studies to inhibit cell proliferation.[17,18,32] The pretreatment of hepatocyte monolayers with MMC for 2 hours resulted in a dose-dependent inhibition of bFGF-stimulated BrdU uptake at the wound front (Fig 2). At an MMC concentration of 4 μg/mL, the BrdU uptake was reduced to a basal level of 3.4%, indicating inhibition of bFGF-stimulated cell proliferation. (In all of the following migration experiments, hepatocyte monolayers were pretreated with 4 μg/mL of MMC before wound formation to inhibit cell proliferation.) The addition of varying doses of bFGF resulted in a concentration-dependent increase in hepatocyte migration at the wound front (Fig 3), indicating that bFGF has a direct stimulatory effect on hepatocyte migration. The effect of bFGF reached a plateau level at a concentration of 30 ng/mL. (In subsequent experiments a bFGF concentration of 30 ng/mL was used to maintain experimental consistency.)

bFGF modulation of clone 9 actin stress fibers. Previous studies have suggested a central role for actin stress fibers in the migration of intestinal epithelial cells.[16,32,33] In the following studies we examined the effect of bFGF on actin stress fibers in hepatocytes. The actin stress fibers were stained with fluorescent-labeled phalloidin (Fig 4). Following an excisional wound, there was an increase was seen in the distribution of actin stress fibers in the cells at the migration front (Fig 4, *A*). As shown in Fig 4, *B*, bFGF (30 ng/mL) treatment resulted in a significant increase in actin stress fibers in the cells at the wound front. Quantitation of fluorescein-labeled actin filaments revealed an approximate 150% increase in actin filaments in the bFGF-treated cells (Fig 4, *E*). The bFGF-induced increase in actin-stress fiber formation correlated with increased stretching and migration of the hepatocytes into the wound site.

Effect of PKA activation on bFGF-stimulated hepatocyte migration. In the following experiments the possible modulatory action of PKA signaling pathways on bFGF stimulation of hepatocyte migration during wound re-epithelialization was examined. To determine the effect of PKA activation on hepatocyte migration, the effect of various agents that activate PKA at different levels of cAMP amplification cascade was studied. The addition of 8Br-cAMP (membrane-permeable cAMP) to the wounded hepatocyte monolayers resulted in a concentration-dependent inhibition of bFGF-stimulated cell migration (Fig 5). The addition of IBMX (phosphodiesterase inhibitor) and forskolin (adenylate cyclase activator) also resulted in a dose-dependent inhibition of bFGF-induced increase in hepatocyte migration (Figs 6 and 7). The stimulatory G-protein activator cholera toxin also inhibited bFGF-stimulated increase in cell migration (Fig 8). Protein kinase A (PKA) activating agents including IBMX and forskolin also inhibited basal migration rates of control monolayers to a similar extent (data not shown).

Because bFGF stimulation of hepatocyte migration was associated with an increase in actin stress fibers in the migrating cells at the wound front, the effect of PKA activating agents on bFGF-stimulated increase in actin stress fibers was also examined. Forskolin, IBMX, and cholera toxin almost completely abolished bFGF-stimulated increase in actin stress fiber formation (Fig 4, *C, D, E*). The downregulation of actin stress fiber formation was accompanied by the absence of cell stretching at the wound front.

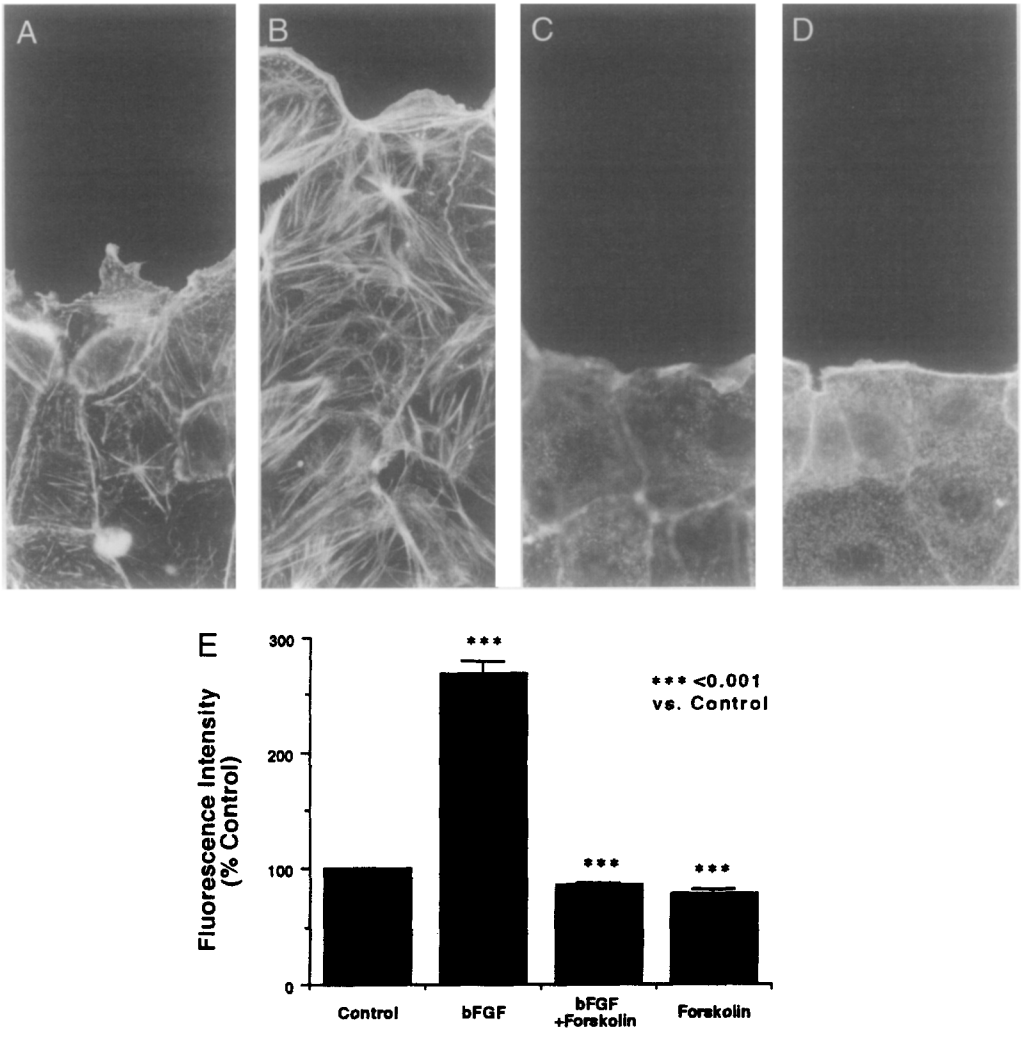

Fig 4. Photomicrographs illustrating localization of hepatocyte actin microfilaments at wound margin. Clone 9 hepatocyte actin microfilaments were labeled with fluorescein–conjugated phalloidin as described in Material and Methods section. Immunofluorescent localization of actin microfilaments in **(A)** control, **(B)** bFGF (30 ng/mL), **(C)** forskolin (150 μmol/L) and bFGF (30 ng/mL), and **(D)** forskolin (150 μmol/L)-treated monolayers at wound front. **E,** Fluorescent intensity of labeled actin microfilaments quantitated with computerized image analysis system. Relative fluorescent intensity of labeled actin stress fibers was quantitated with Nikon PCM 2000 confocal microscope with C-IMAGING-image analysis system. Values represent fluorescent intensity (mean ± SEM) obtained from 3 separate experiments. bFGF treatment resulted in increased formation of actin stress fibers in migrating cells at wound front. Forskolin inhibited formation of actin stress fibers by hepatocytes and prevented stretching of hepatocytes at wound front. Data are representative of results obtained from 3 separate experiments.

Effect of PKA activation agents on hepatocyte proliferation. In the following studies the possible effect of PKA activating agents on bFGF-stimulated hepatocyte proliferation was examined by measuring [^{3}H]-thymidine uptake by the proliferating clone 9 cells. bFGF (30 ng/mL) treatment resulted in a significant increase in [^{3}H]-thymidine uptake by the proliferating hepatocytes (Fig 9). IBMX (1 mmol/L) and forskolin (150 μmol/L) significantly inhibited both basal (data not shown) and bFGF-stimulated increase in [^{3}H]-thymidine uptake by the hepatocytes (Fig 9). These findings suggested that activation of PKA signaling pathways inhibits both basal and bFGF-stimulated proliferation of clone 9 cells.

Effect of extracellular matrix components on bFGF–stimulated hepatocyte migration. Following liver injury, there is a marked increase in the synthesis of various components of ECM.[21,34] Previous studies have shown that specific components of ECM have selective modulatory action on the migration of intestinal

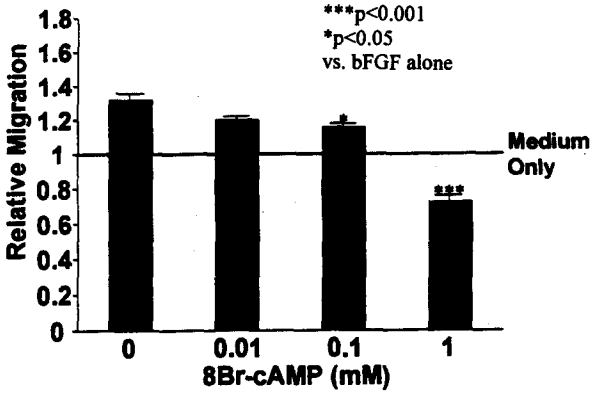

Fig 5. Effect of varying concentration (0, 0.01, 0.1, 1 mmol/L) of 8Br-cAMP on bFGF (30 ng/mL)-induced stimulation of hepatocyte migration. Area of migration was expressed as relative migration or fractional migration (mean ± SEM) of control monolayers (n = 4). 8Br-cAMP concentration of 0 mmol/L represents "bFGF alone." Relative migration of control monolayers (medium alone) were assigned value of 1.0. Data are representative of results obtained from 3 separate experiments.

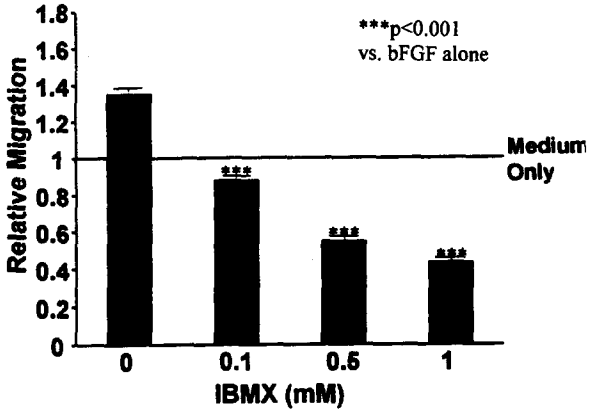

Fig 6. Effect of varying concentration of IBMX on bFGF (30 ng/mL)-induced stimulation of hepatocyte migration. Area of migration was expressed as fractional migration (mean ± SEM) of control monolayers (n = 4). Data are representative of results obtained from 4 separate experiments.

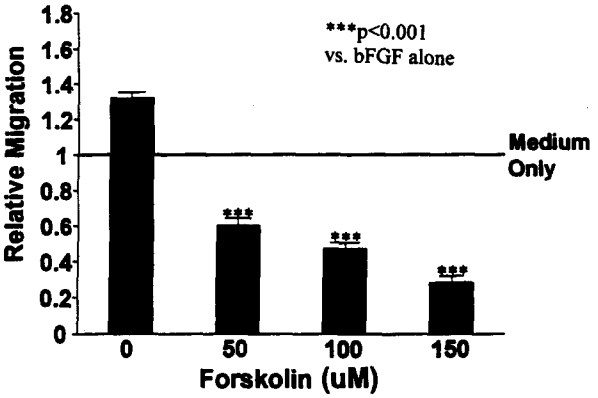

Fig 7. Effect of varying concentration of forskolin on bFGF (30 ng/mL)-induced enhancement of hepatocyte migration. Area of migration was expressed as fractional migration (mean ± SEM) of control monolayers (n = 4). Data are representative of results obtained from 3 separate experiments.

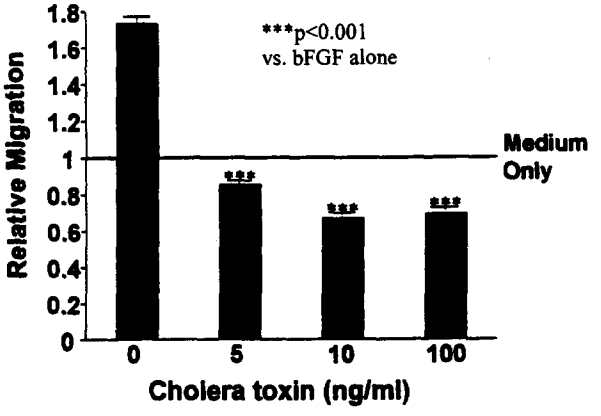

Fig 8. Effect of varying concentrations of cholera toxin on bFGF (30 ng/mL)-induced enhancement of hepatocyte cell migration. Area of migration was expressed as fractional migration (mean ± SEM) of control monolayers (n = 4). Data are representative of results obtained from 3 separate experiments.

epithelial cell.[32] In the following studies the effect of various components of ECM on basal and bFGF-stimulated hepatocyte migration was examined. The basement membrane was coated with specific components of ECM including collagen I, collagen IV, laminin, or fibronectin. The basement membrane coating with selected components of ECM resulted in an ECM–dependent enhancement of basal migration rates (collagen IV ≥ laminin > collagen I > fibronectin > plastic or control) (Fig 10). Varying the composition of ECM did not have a significant modulatory effect on bFGF-induced increase in hepatocyte migration (Fig 10). bFGF produced a similar increase (~60%) in hepatocyte migration regardless of the coated ECM. In contrast, PKA inhibition of hepatocyte migration was dependent on the coated ECM. The PKA activating agent IBMX produced inhibition of hepatocyte migration across all ECM-coated surfaces studied (Fig 10), but IBMX had a significantly greater inhibitory effect on hepatocyte migration on collagen type IV-, fibronectin-, and laminin-coated surface ($P < .05$) compared with the collagen type I-coated or plastic (control) surface.

Effect of bFGF on hepatocyte bFGF receptor-1 protein. In the following studies the possible regulatory effect of bFGF on the bFGF-receptor protein level was examined. The bFGF receptor protein level was assessed by

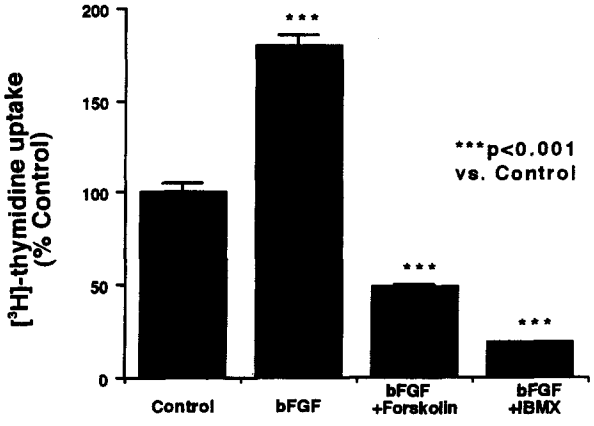

Fig 9. Effect of PKA-activating agents on bFGF-stimulation of [^{3}H]-thymidine uptake. Uptake of [^{3}H]-thymidine was determined as described in Material and Methods. Clone 9 hepatocytes grown in 24-well plates were treated with bFGF (30 ng/mL), bFGF (30 ng/mL), IBMX (1 mmol/L), bFGF (30 ng/mL), and forskolin (150 μmol/L). Values represent percent [^{3}H]-thymidine uptake (mean ± SEM) of control cells (n = 4). Data are representative of results obtained from 3 separate experiments.

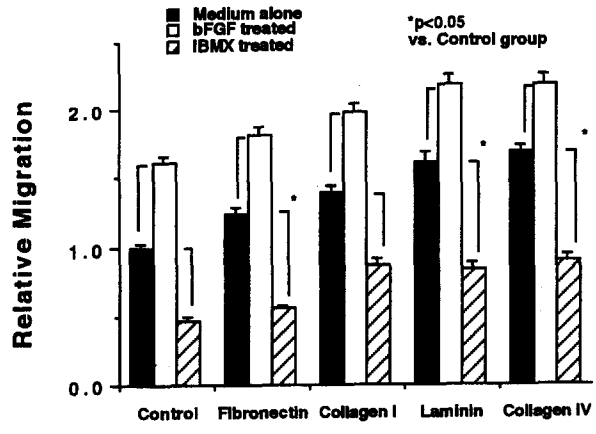

Fig 10. Effect of ECM on bFGF–induced enhancement of rat hepatocyte migration 6-well plates were coated with selected components of ECM as described in Methods. Effect of selected components of ECM on basal (controls) hepatocyte migration (mean ± SEM). Effect of bFGF (30 ng/mL) on ECM modulation of hepatocyte migration is represented by opened bars. Effect of IBMX (1 mmol/L) on ECM modulation of hepatocyte migration is shown. Experiments were repeated 5 times to ensure reproducibility.

Western blot analysis of bFGF receptor-1 protein. bFGF treatment (30 ng/mL) resulted in a significant increase (~90%) in the bFGF receptor-1 protein level (Fig 11). Varying the ECM composition did not significantly affect the basal levels of bFGF receptor-1 protein levels. bFGF treatment produced a similar increase in the bFGF-1 receptor protein level across all basement membrane-coated surfaces examined (Fig 11). These findings suggest the bFGF produces an upregulation of

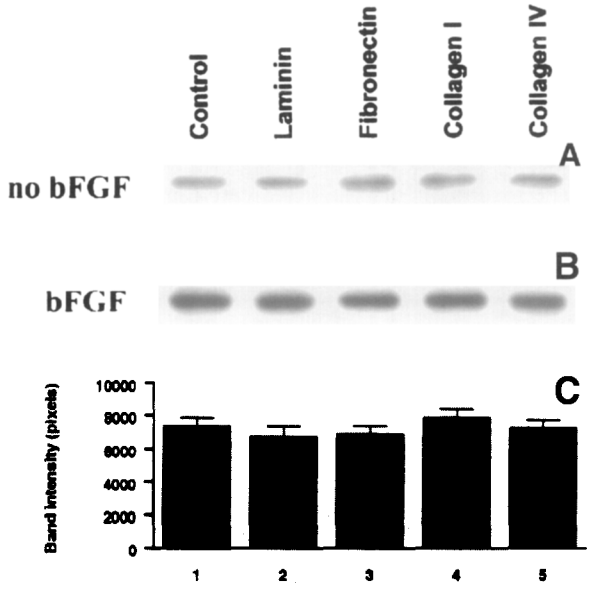

Fig 11. Western blot analysis demonstrating effect of bFGF (30 ng/mL) and ECM components on hepatocyte bFGF receptor-1 protein expression. Hepatocyte monolayers were grown on either plastic (control), laminin, fibronectin, collagen type I, or collagen type IV-coated surface as described in Material and Methods. Subsequently, hepatocyte monolayers were incubated with either F12K media alone or F12K media containing bFGF (30 ng/mL). After 24-hour incubation, hepatocyte monolayers were lysed and Western blot analysis performed. **A,** Effect of varying basement membrane composition on bFGF receptor-1 protein level. **B,** Effect of bFGF (30 ng/mL) on bFGF receptor-1 protein level in hepatocytes grown on various ECM. **C,** Densitometry measurements of bFGF receptor-1 protein bands after exposure to bFGF (30 ng/mL) for 10 minutes expressed in pixels. Results are presented as mean ± SEM obtained from 3 separate experiments.

the hepatocyte bFGF-receptor-1 protein, whereas basement membrane composition did not affect the bFGF-receptor-1 protein level.

DISCUSSION

The 2 major repair processes after wounds to the liver occur are proliferation and migration of the nearby hepatocytes. Shortly after liver injury occurs, hepatocytes rapidly migrate to the site of the wound in a restitutive repair response. Some of the important factors that have been identified in liver wound healing include production of growth factors,[2,3] formation of specific components of ECM,[21] and activation of various intracellular signaling pathways.[22] It has been demonstrated that after liver injury occurs, bFGF production is markedly increased.[1,35] Although bFGF is likely to play an important role in the repair of liver injury, its precise action on repair of the epithelial wound remains unclear. In this study we tested the hypothesis that

bFGF accelerates healing of wounded hepatocyte monolayers by stimulating both cell proliferation and migration with an in vitro wound model. We also examined the modulatory role of intracellular signaling pathways and ECM components on bFGF stimulation of hepatocyte migration.

The results of this study indicate that bFGF stimulates repair of wounded hepatocyte monolayers by enhancing both hepatocyte proliferation and migration. bFGF treatment (30 ng/mL) produced a significant increase (~150%) in BrdU uptake by the hepatocytes at the wound margin, indicating stimulation of hepatocyte proliferation. bFGF also stimulated the migration of the hepatocyte at the wound front. The pretreatment of the hepatocyte monolayers with MMC (4 μg/mL) ensured that the observed re-epithelialization was due to cell migration and not proliferation. This is the first study to demonstrate that bFGF stimulates proliferation and migration of the hepatocyte after wound formation. Our findings suggest that increased production of bFGF after liver injury[1,35] is likely to be beneficial in accelerating liver epithelial wound repair.

Our findings also suggest that the cellular mechanism of bFGF-stimulated increase in hepatocyte migration may be dependent on upregulation of the actin stress fibers. A direct structural-functional correlation was found between bFGF stimulation of actin stress fiber formation and an increase in stretching and migrating of the cells at the wound front. In a converse manner, PKA-activating agents almost completely inhibited bFGF-induced stimulation of actin stress fiber formation and prevented bFGF-induced stretching and migration of the cells at the wound margin. These findings suggest that bFGF stimulation and PKA modulation of the bFGF effect on hepatocyte migration may be related to their action on actin stress fibers.

Previous studies have demonstrated the importance of various intracellular signaling pathways in the modulation of various cellular functions including ion secretion, cytoskeletal rearrangement, transport of various substrates, and cellular migration.[23-27] Our results indicate that bFGF–induced stimulation of hepatocyte migration into the wound site could be abolished by the activation of the PKA pathway. The treatment of wounded hepatocyte monolayers with selected PKA activating agents, which act at different levels of cAMP amplification cascade, prevented both basal and bFGF stimulated increases in epithelial migration. In contrast, PKC activating agents did not have any effect on basal or bFGF-stimulated increases in hepatocyte migration. Consistent with these findings, Manske et al[28] also reported that activation of PKA signaling pathways interfered with epidermal growth factor enhancement of rat hepatocyte migration. These findings in combination suggest that activation of PKA signaling pathways downregulates both basal and bFGF-stimulated migration. In addition to inhibiting epithelial migration, PKA-activating agents also inhibited proliferation of clone 9 hepatocytes (Fig 9). Thus it appears that activation of the PKA signaling pathway could interfere with hepatocyte wound repair by inhibiting both the hepatocyte migration and the hepatocyte proliferation.

After both acute and chronic liver injury, synthesis of ECM components is markedly increased.[17,29-31] Specific components of ECM have been shown to modulate migration of the intestinal epithelial cells.[32] These findings also indicate that coating of the basement membrane with specific components of ECM can selectively stimulate basal hepatocyte migration rates. Our results indicated that collagen type IV and laminin stimulated basal migration of the hepatocyte significantly more than fibronectin or collagen type I. Our findings suggested that the increased synthesis of specific components of ECM that occurs after liver injury[21,34] could play a role in facilitating hepatocyte migration and wound repair. bFGF produced an additive effect on ECM enhancement of hepatocyte migration, suggesting that these 2 factors in combination optimize hepatocyte migration into the wound site.

In summary, bFGF stimulates both migration and proliferation of the clone 9 hepatocytes after excisional wound formation. bFGF stimulation of hepatocyte migration paralleled actin stress fiber formation. The PKA inhibition of bFGF-stimulated increase in hepatocyte migration correlated with PKA downregulation of actin stress fiber formation. The basement membrane composition of the ECM had a selective enhancing effect on basal hepatocyte migration, and bFGF had an additive effect on ECM enhancement of hepatocyte migration. The results of this study provide evidence that bFGF production after liver injury could play an important role in liver wound repair by stimulating both proliferation and migration of the hepatocyte at the margin of the wound injury.

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