CXCL12 induces hepatic stellate cell contraction through a calcium-independent pathway

Yedidya Saiman, Ritu Agarwal, DaShawn A. Hickman, Michel Fausther, Ahmed El-Shamy, Jonathan A. Dranoff, Scott L. Friedman, and Meena B. Bansal

1Division of Liver Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York; 2Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas; and 3Department of Virology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

Submitted 3 May 2012; accepted in final form 8 June 2013

Saiman Y, Agarwal R, Hickman DA, Fausther M, El-Shamy A, Dranoff JA, Friedman SL, Bansal MB. CXCL12 induces hepatic stellate cell contraction through a calcium-independent pathway. Am J Physiol Gastrointest Liver Physiol 305: G375–G382, 2013. First published June 27, 2013; doi:10.1152/ajpgi.00185.2012.—Liver fibrosis, with subsequent development of cirrhosis and ultimately portal hypertension, results in the death of patients with end-stage liver disease if liver transplantation is not performed. Hepatic stellate cells (HSCs), central mediators of liver fibrosis, resemble tissue pericytes and regulate intrahepatic blood flow by modulating pericapillary resistance. Therefore, HSCs can contribute to portal hypertension in patients with chronic liver disease (CLD). We have previously demonstrated that activated HSCs express functional chemokine receptor, CXCR4, and that receptor engagement by its ligand, CXCL12, is increased in patients with CLD, leads to further stellate cell activation in a CXCR4-specific manner. We therefore hypothesized that CXCL12 promotes HSC contraction in a CXCR4-dependent manner. Stimulation of HSCs on collagen gel lattices with CXCL12 led to gel contraction and myosin light chain (MLC) phosphorylation, which was blocked by addition of AMD3100, a CXCR4 small molecule inhibitor. These effects were further mediated by the Rho kinase pathway since both Rho kinase knockdown or Y-27632, a Rho kinase inhibitor, blocked CXCL12 induced phosphorylation of MLC and gel contraction. BAPTA-AM, a calcium chelator, had no effect, indicating that this pathway is calcium sensitive but not calcium dependent. In conclusion, CXCL12 promotes stellate cell contractility in a predominantly calcium-independent fashion. Our data demonstrates a novel role of CXCL12 in stellate cell contraction and the availability of small molecule inhibitors of the CXCL12/CXCR4 axis justifies further investigation into its potential as therapeutic target for portal hypertension.

liver fibrosis; CXCR4; portal hypertension

In the United States, end-stage liver disease is the fifth leading cause of death among people during their most productive phase of life (45- to 64-year age group (National Vital Statistics Report 2011)). In these patients, complications associated with portal hypertension, including ascites, hepatic encephalopathy, and hemorrhage from gastroesophageal varices are the main causes of death (5). In the normal liver, hepatic stellate cells (HSCs) are quiescent, vitamin A-rich cells located between sinusoidal endothelial cells and hepatocytes in the space of Disse (9). During injury, these quiescent cells differentiate into highly proliferative and contractile fibrogenic cells that increase expression of contractile proteins including α-smooth muscle actin (α-SMA). HSCs additionally resemble tissue pericytes, a cell population that has smooth muscle features and is thought to regulate blood flow by modulating pericapillary resistance (22). They contract to factors such as endothelin-1 (ET-1) and angiotensin, which are elevated during liver injury implicating stellate cell contraction in the pathogenesis of portal hypertension (3, 14). In all etiologies of liver disease, hepatic expression of CXCR4 and both serum and hepatic levels of CXCL12 are increased (12, 25). In the liver, CXCL12 is expressed by bile duct epithelia, sinusoidal endothelial cells, and HSCs; however, the function of this chemokine in liver disease is not well understood (12, 20, 25). We have previously reported that activated HSCs express functional CXCR4 and that receptor engagement by its ligand, CXCL12, leads to a profibrogenic phenotype characterized by increased proliferative capacity and collagen type I and α-SMA production (12). Furthermore, others have shown that CXCL12 promotes HSC migration and chemotaxis (20). Here we show that CXCL12 induces cellular contraction and myosin light chain phosphorylation in a CXCR4 and Rho kinase-dependent manner.

MATERIALS AND METHODS

Cell lines. All experiments were performed with JS1 cells unless otherwise noted. JS1 cells are a murine SV40 immortalized HSC line with a highly activated phenotype (11). For other experiments (where stated), the LX2 spontaneously immortalized human stellate cell line was used (10).

Isolation of primary human HSCs. Primary stellate cells were isolated from wedge sections of normal human liver in patients undergoing hepatic resection for primary benign tumors or single metastasis from colon cancer as previously described (12). These studies have been approved by the Mount Sinai IRB and designated exempt category 4 (GCO no. 06-0523). Briefly, immediately after hepatectomy, the liver was placed in DMEM. The liver was washed and portal venules cannulated for in situ digestion with pronase (Roche Applied Sciences, Mannheim, Germany) and collagenase B (Roche Applied Sciences). Stellate cells were isolated via density centrifugation and plated on plastic in DMEM supplemented with 10% FBS. HSCs were activated by culturing on plastic for 7–12 days and subcultured to passage 3 for all experiments.

Collagen gel contraction assay. The ability of HSCs to contract in response to CXCL12 was evaluated by using collagen gel lattices in 24-well culture plates as previously described (19). The 24-well plates were incubated with 1% sterile BSA in PBS for 1 h at 37°C, washed twice with PBS, and air dried. Eight parts type 1 bovine collagen, PureCol (Advanced BioMatrix, San Diego, CA), were added to 1 part 10× MEM (GIBCO, Grand Island, NY) and 1 part 0.2 M HEPES.
(resulting in a final collagen concentration of 2.4 mg/ml), pH adjusted to 7.4, and 500 μl added to each well. Collagen gels were allowed to solidify at 37°C for 1 h. After gelation, 1 × 10^5 JS1, LX2 or primary human stellate cells in 1 ml 10% FBS-1% P/S DMEM media were added on top of the gels and incubated for 24 h. Cells were serum starved in 0.2% BSA DMEM overnight. Gels were detached from the plates and treated for 24 h with species-specific recombinant CXCL12 (R&D Systems, Minneapolis, MN) or synthetic human ET-1 (Sigma, St. Louis, MO). For Rho kinase or CXCR4 inhibition, serum-free medium containing 25 μM Y-27632 (EMD Chemicals) or 5 μg/ml AMD3100 (Sigma), respectively, was added to the cells for 30 min prior to adding CXCL12. For analysis, gels were scanned and area was measured by use of digital image analysis software. All experiments were performed in triplicate.

**Immunoblots.** Expression of α-SMA and phosphorylated myosin light chain 2 (pMLC-2) was examined in JS1 and LX2 cells. Briefly, 10^5 JS1, LX2, or primary human stellate cells were plated in six-well plates and after 24 h serum starved overnight in 0.2% BSA DMEM. Cells were stimulated with species-appropriate recombinant CXCL12 (0–750 ng/ml), for 1–24 h. Cells were further treated with 25 ng/ml murine recombinant CCL5 (R&D Systems), which is the optimal concentration to promote stellate cell migration and proliferation (21), or 2 × 10^-8 synthetic human ET-1. For experiments with Rho kinase or CXCR4 inhibition cells were preincubated with 25 μM Y-27632 or 5 μg/ml AMD3100, respectively, for 30 min, followed by addition of CXCL12. Then 40 μg of cellular protein in RIPA buffer was separated by SDS-PAGE (4–12% gradient gel, Invitrogen), transferred to PVDF membrane, blocked overnight in 5% BSA PBS-T, and probed for α-SMA or pMLC-2 for 48 h. For loading control of pMLC-2, the pMLC-2 antibody was stripped from the membrane and the membrane was reprobed for MLC-2. The following antibodies with corresponding dilutions were used: rabbit anti-mouse polyclonal α-SMA (1:1,000; Abcam, Cambridge, MA); rabbit anti-mouse polyclonal phosho-MLC-2 (1:1,000 Cell Signaling Technology, Danvers, MA); rabbit anti-mouse polyclonal MLC-2 (1:1,000 Cell Signaling Technology); rabbit anti-mouse polyclonal CXCR4 (1:500 Abcam); rabbit anti-mouse monoclonal ROCK1 (1:500 Cell Signaling Technology); and rabbit anti-mouse polyclonal GAPDH (1:3,000 Santa Cruz Biotechnology); species-appropriate HRP-conjugated secondary antibodies were used at 1:1,000–1:3,000 dilutions and blots were developed by use of Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA).

**ELISA for cell supernatant CXCL12.** To evaluate whether JS1 cells secrete CXCL12, ELISA was performed on conditioned media from JS1 cells. We plated 2 × 10^4 JS1 cells in 24-well plates; after 24 h growth, 300 μl of fresh media added. Cell culture supernatant was

---

**Fig. 1.** JS1 cells express CXCR4 and CXCL12. FACS analysis for CXCR4 conjugated to allophycocyanin (APC) or isotype control demonstrates both intercellular and cell surface CXCR4 protein expression. For cell surface expression JS1 cells were vigorously pipetted in PBS prior to staining with APC-conjugated anti-CXCR4 for 1 h at 4°C. For intercellular staining cells were collected with trypsin, fixed, and permeabilized and stained for 1 h at room temperature with anti-CXCR4. Representative FACS analyses from 3 independent experiments are shown (A). Immunofluorescence staining on JS1 reflects expression of CXCR4 in green (B). Nuclei were stained blue with DAPI. ELISA for CXCL12 performed on 0- to 96-h conditioned media from JS1 cells reveals significant secretion of CXCL12 (>6,000 pg/ml) (C). Medium incubated without cells was used as a negative control. Purified recombinant human CXCL12 was used to generate a standard curve. CXCL12 concentrations are an average of 3 independent experiments.
CXCL12 PROMOTES HEPATIC STELLATE CELL CONTRACTION

JS1 cells, a murine HSC line, express CXCR4 and CXCL12. We have previously reported that activated human HSCs express both the CXCR4 receptor and its ligand, CXCL12 (12). To determine whether JS1 cells express CXCR4, we performed FACS analysis for both total and cell surface expression and

FACS analysis for cell surface and total CXCR4. FACS analysis was performed to demonstrate both cell surface and total CXCR4 expression by JS1 cells. For cell surface expression, cells were collected by vigorous pipetting in PBS. Non-specific binding was blocked by incubating cells in 1:200 dilution mouse serum (Sigma) for 30 min followed by addition of 1:100 rat anti-mouse monoclonal CXCR4 conjugated to APC (BD Pharmingen, Clone 2B11) or Rat IgG2b isotype conjugated to APC (BD Pharmingen) for 1 h. Cells were washed in FACS buffer and samples analyzed on a BD LSRII (BD Biosciences). For total CXCR4 expression, JS1 cells were fixed and permeabilized with IC fixation and permeabilization buffer (eBiosciences) followed by blocking and incubation with CXCR4-APC or RatIgG2b-APC for 1 h. For TGF-β1 stimulation, cells were treated with hTGF-β1 for 2 h prior to collection and staining.

Immunostaining for CXCR4. For the detection of CXCR4 on stellate slides, fixed in 4% PFA for 10 min, permeabilized with 0.5% Triton/PBS for 20 min at 37°C, and blocked for 1 h with blocking solution (PBS with 1% bovine serum albumin, 0.1% Triton). Subsequently, cells were incubated with rabbit anti-mouse polyclonal CXCR4 at 1:100 dilution (eBiosciences) or isotype control. Slides were washed in PBS and incubated for 30 min at room temperature with goat anti-rabbit AlexaFluor 488 (Molecular Probes, Grand Island, NY) at a 1:100 dilution. The cells were mounted with ProLong Gold mounting media containing DAPI (Molecular Probes). Images were acquired with a Zeiss Axioskop 2 fluorescence microscope (Zeiss, Oberkochen, Germany).

Calcium influx assay. Changes in cytosolic calcium were determined with confocal video microscopy performed with live human LX2 or murine JS1 stellate cells grown on glass coverslips. Cells were loaded with the calcium-sensitive fluorophore Fluo-4 AM (Molecular Probes) for 20 min at 37°C and mounted on a specially designed stage for use on a confocal microscope, prior to recording. Cells were perfused with HEPES buffer, followed by treatment with HEPES buffer containing CXCL12 (750 250 ng/ml) or ATP (100 μM). Changes in Fluo-4 fluorescence were monitored by use of a Zeiss LSM 510 (Zeiss, Thornwood, NY) confocal imaging system. Fluo-4 fluorescence was excited with a krypton-argon laser at 488 nm; emitted fluorescence at >515 nm was collected. Changes in fluorescence over time were expressed as peak fluorescence divided by or subtracted to initial fluorescence.

Knockdown of CXCR4 and ROCK1 in JS1 cells. JS1 cells were grown to 80% confluence and transfected with HuSH shRNA for CXCR4 (cat. no. TR30007, Origene, Rockville, MD) or SureSilencing shRNA for ROCK1 (cat. no. KM04660G, SABiosciences, Valencia, CA). After 24 h, cells were trypsinized and 105 cells subcultured into six-well plates. Cells were allowed to grow for 24 h, serum starved overnight, and treated as mentioned above and knockdown confirmed by Western blot analysis.

Measurement of cell viability. Cell viability was measured with the MTT Cell Growth Assay Kit (Millipore). We seeded 3 × 104 JS1 cells in a 96-well plate for 24 h. Cells were serum starved overnight and treated with 25 μM Y-27632 for 2.5 h, and cell viability was measured according to manufacturer’s protocol.

Statistics. All results are expressed as means ± SD. Statistical significance was tested by an unpaired Student’s t-test, and P < 0.05 indicated a significant difference.

RESULTS

JS1 cells, a murine HSC line, express CXCR4 and CXCL12.
immunofluorescent staining (Fig. 1, A and B). FACS analysis demonstrates that 100% of JS1 cells express CXCR4 while 21% express it on their cell surface. This is similar to neutrophils, dendritic cells, and macrophages, which contain intracellular cytoplasmic endosomes of CXCR4 that are able to localize to the cell surface upon stimulation (4, 7, 18, 26). To determine whether JS1 cells express the CXCR4 ligand, CXCL12, we performed ELISA on cell culture supernatant from JS1 cells collected between 0 and 96 h (Fig. 1C). There is a time-dependent increase in the secretion and accumulation of CXCL12 and by 96 h the concentration was over 6,000 pg/ml.

CXCL12 leads to upregulation of α-SMA, phosphorylation of MLC, and HSC contraction. Because CXCL12 promotes an activated phenotype in HSCs, we tested whether stimulation with exogenous recombinant CXCL12 leads to increased expression of α-SMA, a critical protein in contraction. JS1 cells were treated with increasing concentrations of recombinant murine CXCL12 for 24 h and Western blot analysis performed for α-SMA (Fig. 2A). Maximum expression of α-SMA was seen with a dose of 250 ng/ml CXCL12; therefore, this dosage was chosen for all other experiments. The mechanism of cellular contraction is well described, and the final step requires the phosphorylation of myosin light chain 2 (MLC-2) and we show that CXCL12 leads to an increase in the phosphorylation of MLC-2. JS1 cells were treated with 250 ng/ml of CXCL12 for 0–8 h and Western blot analysis performed with an antibody that recognizes the phosphorylated form of MLC-2 (Fig. 2B). At 2 h after treatment we saw the greatest increase in MLC-2 phosphorylation. To determine that CXCL12 can also lead to stellate cell contraction we treated JS1 cells plated on collagen lattices with 250 ng/ml CXCL12 for 24 h. Treatment of collagen gels with CXCL12

---

**Fig. 3.** The human hepatic stellate cell (HSC) line LX2 and primary human HSCs contract in response to CXCL12. To demonstrate that CXCL12-induced contraction is relevant to human stellate cells we treated LX2 cells and primary human HSCs with CXCL12. LX2 cells treated with 750 ng/ml human CXCL12 (A) or 2 × 10⁻⁸ M endothelin-1 (ET-1; B) show a time-dependent increase in phosphorylation of MLC-2. LX2 cells seeded on collagen gels and treated with 750 ng/ml CXCL12 for 24 h shows gel contraction (C) (representative image). SFM, serum-free medium. Quantification of LX2 collagen gel contraction in response to CXCL12 and ET-1 (D). Similarly, primary human stellate cells treated with 750 ng/ml human CXCL12 or 2 × 10⁻⁸ M ET-1 show an increase in MLC-2 phosphorylation (E) and collagen gel contraction (F and G). *P < 0.05, **P < 0.005, ***P < 0.001; n = 6 from 3 independent experiments.
led to a 21% decrease in collagen gel area (Fig. 2, C and D). Interestingly, even without addition of exogenous CXCL12 there was significant MLC-2 phosphorylation and a 13% contraction of collagen gels. As HSCs express both CXCR4 and its ligand CXCL12, they form a potential autocrine loop capable of inducing contraction and therefore exhibit a high level of basal contraction. Finally, to show that stellate cell contraction is not a general chemokine phenomenon, but specific to CXCL12, we show that there is no increase in MLC-2 phosphorylation after treatment with 25 ng/ml CCL5 (Fig. 2E).

CXCL12 leads to MLC phosphorylation and HSC contraction in human HSCs. To show that CXCL12 also induces contraction in human HSCs we used the LX2 cell line, a human immortalized stellate cell line and primary human HSCs (hHSCs). We have previously shown that both LX2 cells and hHSCs express CXCR4 and CXCL12 and stimulation with exogenous human CXCL12 leads to an increase in α-SMA expression and measured relative change in fluorescence using confocal microscopy. In human LX2 cells treatment with CXCL12 led to a nearly twofold increase in relative fluorescence (Fig. 5, A and B) whereas in murine JS1 cells CXCL12 did not promote calcium transience (Fig. 5C). Despite the effect of CXCL12 on calcium flux in human LX2 cells, pretreatment of either LX2 (Fig. 5D) or JS1 (Fig. 5E) cells with 50 μM BAPTA-AM, an intracellular calcium chelator, did not affect the extent of CXCL12 mediated MLC phosphorylation, indicating that the mechanism of action is not calcium dependent.

CXCL12-dependent contraction is mediated through the Rho kinase pathway. To demonstrate that CXCL12-dependent contraction signals through Rho kinase, we used shRNA to knock down ROCK1 (Rho-associated protein kinase 1), a Rho kinase isoform shown to be important in MLC phosphorylation (16). JS1 cells were transfected with either shSCR or ROCK1-targeted shRNA. Western blot analysis shows that treatment of JS1 cells with 250 ng/ml CXCL12 after ROCK1 knockdown fails to induce MLC phosphorylation (Fig. 6A). Additionally, we show that pretreatment with the Rho kinase inhibitor ROCK1 inhibitor does not prevent JS1 cell contraction in response to CXCL12 (Fig. 6B). Pretreatment with ROCK1 inhibitor reduces collagen gel contraction (C). Quantification of collagen gel contraction with AMD3100 (D). **P < 0.005; n = 6 from 3 independent experiments.
Y-27632 for 30 min followed by stimulation with 250 ng/ml CXCL12 fully abrogated CXCL12-induced MLC phosphorylation (Fig. 6B) and that treatment of JS1 cells on collagen lattices with Y-27632 also abolished gel contraction (Fig. 6C and D) whereas Y-27632 treatment did not affect cell viability as measured by MTT assay (Fig. 6E).

DISCUSSION

In this study we show that HSCs contract in response to CXCL12 through CXCR4 in a Rho kinase-dependent mechanism. We have previously reported that CXCL12 leads to an increased fibrogenic phenotype and now show an additional role for CXCL12 in stellate cell biology. Quiescent stellate cells do not express cell surface CXCR4; however, with activation during liver injury stellate cells begin to express CXCR4 and acquire a profibrogenic profile. In the normal liver CXCL12, the CXCR4 ligand, is predominantly expressed by biliary epithelial cells (BECs) (25). During progressive injury there is an anatomical and cellular redistribution of CXCL12 to proliferating bile ducts and to cells of the fibrotic septae including sinusoidal endothelial cells and HSCs (12). We show that stellate cells express functional cell surface and intracellular CXCR4 and secrete high levels of CXCL12, allowing for both paracrine (from BECs and liver sinusoidal endothelial cells) and autocrine stimulation. Activation of both human and murine stellate cells with exogenous CXCL12 led to phosphorylation of MLC-2 and stellate cell contraction to a similar extent of ET-1. The dose of CXCL12 required for maximal contraction in LX2 cells and hHSCs is greater than that of JS1 cells, 750 ng/ml vs. 250 ng/ml, respectively, and may be due to the levels of endogenous CXCL12 secreted by primary human stellate and LX2 cells. We have previously shown that LX2 cells and hHSCs cells secrete ~3,000 pg/ml CXCL12 vs. 6,000 pg/ml in JS1 cells (12). This difference may also be responsible for the lower level of basal contraction seen in the human cells since we show that endogenously secreted CXCL12 is responsible for basal MLC-2 phosphorylation as inhibiting CXCR4 decreases pMLC-2 even in the absence of CXCL12 stimulation.

The final step in the induction of cell contraction is phosphorylation of MLC-2. Two mechanistically distinct pathways exist: the calcium-dependent pathway and the calcium-sensitive pathway. In the calcium-dependent pathway, primarily found in skeletal and cardiac muscle, release of calcium from the endoplasmic/sarcoplasmic reticulum leads to activation of MLC kinase by calmodulin and subsequent phosphorylation of MLC. Alternatively, in the calcium-sensitive pathway, active in smooth muscle-like cells, Rho kinase phosphorylates the myosin binding subunit, thereby inactivating it and preventing it from dephosphorylating MLC and inhibiting contraction. The net effect of Rho kinase activation is increased phosphorylated MLC and contraction.
CXCL12 is well known to induce calcium transience in numerous cell types which is important in cellular chemotaxis and migration (2, 15). We show that CXCL12 induces calcium transience in LX2 but not JS1 cells at the CXCL12 concentrations that promoted maximum α-SMA expression in each cell type. Despite this difference, in both LX2 and JS1 cells, preincubation of cells with a calcium chelator did not inhibit CXCL12 induced contraction, indicating that this pathway is not calcium-dependent but signals through the Rho kinase-mediated calcium-sensitive pathway.

The role of Rho kinases in liver disease has been studied extensively and is implicated in activation and contraction of stellate cells. We demonstrate that CXCL12 signals by activating the Rho kinase pathway as stellate cell stimulation with CXCL12 after knockdown of Rho kinase isoform ROCK1 failed to induce MLC-2 phosphorylation. Finally, systemic and stellate cell-specific delivery of Y-27632, a Rho kinase inhibitor, leads to decreased fibrosis in murine models of liver injury (13, 23, 24). Similar to previous reports that HSC contraction occurs through the Rho kinase pathway, we show that pretreatment of stellate cells with Y-27632 abolishes CXCL12-induced contraction.

Despite the identification of vasoactive factors contributing to increased sinusoidal resistance, treatment options for these patients remain limited since inhibiting these pathways leads to systemic vascular effects with minimal hepatic response. The CXCL12/CXCR4 axis is not involved in normal homeostatic vasoregulation, and in healthy tissue vascular smooth muscle cells and pericytes do not express CXCR4. Only during injury do vascular smooth muscle cells begin to express CXCR4 (6). Additionally, in models of cardiac infarction, coronary microvessels specifically from injured tissue, but not healthy controls, contract in response to CXCL12 (17). Blocking the CXCL12/CXCR4 axis in patients with portal hypertension would therefore potentially have liver-specific effects. We show that blocking CXCR4 with AMD3100, which is FDA approved for treatment of multiple myeloma, leads to a decrease in CXCL12-stimulated pMLC-2 in cells knocked down for ROCK1. JS1 cells were transfected with either shControl or ROCK1-targeted shRNA (shROCK1) and stimulated with 250 ng/ml CXCL12 for 2 h to show a decrease in MLC phosphorylation with ROCK1 knockdown. MLC-2 was used as a loading control (A). JS1 cells treated with CXCL12 alone or pretreated with 25 μM Y-27632, a Rho kinase inhibitor, for 30 min, showing that Rho kinase inhibition decreases MLC phosphorylation (B) and abolishes collagen gel contraction (C). Quantification of collagen gel contraction (n = 6 from 3 independent experiments) (D). MTT cell survival analysis was performed on JS1 cells treated with or without 25 μM Y-27632 for 2.5 h. Absorbance at 570 nm shows no difference in cell survival with treatment (E). Western blots and collagen gels are representative images. Experiments were repeated 2–3 independent times. ***P < 0.001. ns, Not significant.

Fig. 6. CXCL12-induced contraction signals through Rho kinase. Western blot for phosphorylated MLC-2 showing a decrease in CXCL12-stimulated pMLC-2 in cells knocked down for ROCK1. JS1 cells were transfected with either shControl or ROCK1-targeted shRNA (shROCK1) and stimulated with 250 ng/ml CXCL12 for 2 h to show a decrease in MLC phosphorylation with ROCK1 knockdown. MLC-2 was used as a loading control (A). JS1 cells treated with CXCL12 alone or pretreated with 25 μM Y-27632, a Rho kinase inhibitor, for 30 min, showing that Rho kinase inhibition decreases MLC phosphorylation (B) and abolishes collagen gel contraction (C). Quantification of collagen gel contraction (n = 6 from 3 independent experiments) (D). MTT cell survival analysis was performed on JS1 cells treated with or without 25 μM Y-27632 for 2.5 h. Absorbance at 570 nm shows no difference in cell survival with treatment (E). Western blots and collagen gels are representative images. Experiments were repeated 2–3 independent times. ***P < 0.001. ns, Not significant.
CXCL12 PROMOTES HEPATIC STELLATE CELL CONTRACTION

CXCR4 justify further investigation into its role as a therapeutic target.

ACKNOWLEDGMENTS

The authors thank Feng Hong, Hsini Chou, and JingJing Jiao for technical assistance.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-6047402, DK-071745, and R56DK-092128 (M. B. Bansal) and DK-090986 (Y. Saiman).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES