Pressure Activates Src-Dependent FAK-Akt and ERK1/2 Signaling Pathways in Rat Hepatic Stellate Cells

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Key Words

Hepatic stellate cells • Pressure • Focal adhesion kinase • Akt • Extracellular signal-regulated kinases 1 and 2 • Rats

Abstract

Background and Aims: Hepatic fibrosis is associated with elevated sinusoidal pressure, which can be transmitted to the hepatic stellate cells (HSCs) in the perisinusoidal space of Disse. Here, we sought to determine the effects of pressure on cellular growth and Src-dependent signaling pathways in the rat HSCs. **Methods:** Cultured rat HSCs were exposed to pressures (0 to 80 mmHg) by using a pressure-inducing apparatus. The proliferation of the cells was determined by a 5-bromo-2'-deoxy-uridine (BrdU) incorporation assay. Reverse transcription-PCR and Western-blot analysis were used to examine the mRNA and protein levels of representative molecules in Src-dependent signaling pathways. **Results:** Pressure at 10 to 20 mmHg applied to the HSCs over 1 h upregulated BrdU incorporation and expression of proliferating cell nuclear antigen (PCNA) and type I collagen, while a higher pressure of 40-80 mmHg did not have noticeable effect. The mRNA level of β_3

integrin was increased by 1-h application of 5 to 20 mmHg. Immunoblot with phospho-specific antibodies demonstrated the phosphorylation of Src (Tyr418), focal adhesion kinase (FAK) (Tyr397), Akt (Ser473) and extracellular signal-regulated kinases 1 and 2 (ERK1/2) (Thr421/Ser424) was increased in response to 10-mmHg pressure. Herbimycin A, an inhibitor of Src phosphorylation, attenuated the pressure-induced HSC proliferation and phosphorylation of above-mentioned signaling molecules. **Conclusion:** Our data demonstrated that pressure alone induced HSC proliferation involving the activation of Src-dependent signaling pathways.

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Introduction

The hepatic stellate cell (HSC), a mesenchymal cell type in the hepatic parenchyma, is the primary cell type in the liver responsible for excess collagen deposition during fibrosis. HSCs are located between hepatic parenchymal cell plates and sinusoidal endothelial cells.

These cells have extensive network of processes that extend to the surrounding sinusoids as pericytes [1]. Normal, quiescent HSCs function as major vitamin A storage site, and contain >80 % of the total vitamin A in the body. Following a fibrogenic stimulus, HSCs proliferate and secrete excess type I collagen [2, 3]. Swelling of hepatocytes and collagen deposition in the space of Disse [4, 5] increase the inflow resistance. Together with an increase in intrahepatic flow due to splanchnic vasodilation [6], these events lead to an eventual elevation in the sinusoidal pressure.

Sinusoidal pressure can be evaluated by wedged hepatic vein pressure, which increases from 5 mmHg under normal conditions to approximately 40 mmHg in liver cirrhosis [7]. Elevated sinusoidal pressure accelerates transsinusoidal fluid flux, and by doing so increases interstitial fluid pressure [8]. Given their anatomical location, HSCs are subjected to the elevated sinusoidal pressure as hepatic fibrosis progresses.

Pressure has been demonstrated an important stimulus for many types of cells, affecting their phenotype, proliferation and differentiation [9-11]. Extracellular mechanical stimuli such as pressure modulate cellular activities via mechanotransduction. Focal adhesion kinase (FAK), a nonreceptor protein tyrosine kinase, is the nodal point of mechanotransduction [12]. When cells in the artery are under pressure, FAK is activated by the tyrosine kinase Src and becomes phosphorylated at Tyr397, resulting in the initiation and propagation of cellular growth [13]. Pressure-induced autophosphorylation of FAK at tyrosine 397 causes a rearrangement of focal adhesions to accommodate increased mechanical load in the myocardium [14]. FAK could also form protein complexes with other signaling molecules to activate downstream signaling molecules including Akt and extracellular signal-regulated kinases 1 and 2 (ERK1/2) [15-17].

There have been few details available about how extracellular pressure induces signal transduction in HSCs although expression of alpha-smooth muscle actin (α -SMA) was found to be upregulated in pressurized HSCs [18]. The phosphatidylinositol 3-kinase (PI3K)-Akt pathway and ERK1/2 pathway have been demonstrated to be important in regulating HSCs proliferation [16]. We hypothesized that Src-dependent signaling pathways may be involved in the response of HSCs to pressure stimulus as hepatic fibrosis development. We sought here to investigate the effects of mechanical pressure on HSC proliferation and the role of Src-dependent signaling pathways in the response of the HSCs to pressure stimulus using an *in vitro* pressure-inducing apparatus.

Materials and Methods

Isolation and culturing of HSCs from rat liver tissue

HSCs were purified from the liver of healthy male Sprague-Dawley rats (weighing approximately 400-600 g) by *in situ* pronase/collagenase digestion of the liver tissue followed by 12% Nycodenz (Axis-Shield PoC, Oslo, Norway) density gradient centrifugation as previously described [19]. The isolated HSCs were cultured at 37°C on uncoated plastic plates containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μ g/ml streptomycin in a humidified atmosphere containing 95% air and 5% CO₂. The medium was replenished every other day. HSCs remained quiescent for 2 days and became fully activated at day 14. The purity of HSCs was assessed by retinoid autofluorescence and immunofluorescence staining using antibodies against desmin (Santa Cruz Biotechnology, Santa Cruz, CA) and α -SMA (Abcam, Cambridge, MA) (data not shown).

The study protocol was approved by the local institutional review board. All animal experiments were performed in compliance with the guidelines by the Second Military Medical University Animal Care and Use Committee.

Pressure loading

The pressure-loading apparatus was set up as described previously with some modifications [10] (Fig. 1). The apparatus consisted of an air-tight steel chamber with inlet and outlet ports. To generate pressure, compressed helium gas was released into the chamber after being preheated through a long tube fixed in the incubator connected to the inlet port. The pressure was confirmed by a sphygmomanometer through a tube connected to the outlet. No prepaced room air was released during the delivery of helium gas into the apparatus and the partial pressure of the gases originally contained in the chamber, including oxygen, nitrogen, and carbon dioxide, were governed by the Boyle-Charles's law [10, 20]. Tissue cultures used for experiments were placed in the chamber inside the incubator. After a 24 h incubation in DMEM containing 0.1% FBS, the cultured cells were exposed to pressure from 0 to 80 mmHg for 1 or 24 h as previously depicted [7].

Cell proliferation assay

Cell proliferation rate was assessed by a 5-bromo-2'-deoxy-uridine (BrdU) incorporation assay. Briefly, HSCs were seeded in a 96-well plate at a density of 5×10^4 cells/well and the adherent cells were starved for 24 h in DMEM containing 0.1% FBS. Then these cells were exposed to pressure from 0 to 80 mmHg for the indicated period of time. After the pressure application, the cells were labeled with 10 μ M of BrdU solution and incubated for an additional 24 h at 37°C. BrdU incorporation was measured by an ELISA kit (eBioscience, San Diego, CA) according to the manufacturer's instructions as described previously [10]. The absorbance of the samples was measured with a microplate reader at 490 nm. After synchronization, the following inhibitors were treated to the HSCs for 1 h before the pressurization: 900 nM herbimycin A (Sigma), 25 μ M PD98059 or 25 μ M LY294002 (Cell Signaling Technology).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from cultured HSCs using the Trizol reagents according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) and the RNA content was measured spectrophotometrically. Two μg of RNA were reverse transcribed to cDNA with 1 μl of oligo(dT) (0.1 $\mu\text{g}/\mu\text{l}$) and 5 μl of 0.1% DEPC H_2O at 70°C for 5 min. The resulting cDNA was amplified with primers specific for β_3 integrin, platelet-derived growth factor (PDGF) receptor, transforming growth factor beta (TGF- β) receptor 1, α -SMA and β -actin, respectively, in a 30- μl reaction mixture containing 167 μM of all four dNTPs, 1.25 U *Taq* NDA polymerase, 3.0 μl 10 x PCR buffer, and 5.0 μl cDNA. Primer sequence is listed in Table 1. PCR was performed using the following thermal cycles: 60 s at 94°C, 60 s at 59°C and 2 min at 72°C for 30 cycles. The PCR products were resolved on 1.5% agarose gel containing 0.004% ethidium bromide and visualized under a UV transilluminator. Densitometric analysis of the PCR products was performed with Image J software and normalized against β -actin.

Western blot analysis

Antibodies against the following proteins were used for Western blotting studies: phospho-Src (Tyr418) (Genway, San Diego, CA), type I collagen, FAK and phospho-FAK (Tyr397) (all from Sigma, St. Louis, MO), PCNA, Akt and phospho-Akt (Ser473), and β -actin (all from Cell Signaling Technology, Beverly, MA), and phospho-Erk1/2 (Thr185/Tyr187) (Millipore, Billerica, MA). Synchronized HSCs were treated with 900 nM herbimycin A (Sigma), 25 μM PD98059 or 25 μM LY294002 (Cell Signaling Technology) for 1 h. The cells were then subjected to the indicated pressures. After wash with phosphate-buffered saline (PBS), the cells were lysed using a lysis buffer containing 100 mM Tris-HCl (pH 6.8), 200 mM dithiothreitol, 4% SDS, 0.2% bromophenol blue, and 20% glycerol. Protein concentration was determined by the bicinchoninic acid method (Pierce, Rockford, IL). The samples were heated at 100°C for 10 min, and 30 μg of each sample was loaded to a 7.5% (for type I collagen) or 10% SDS-polyacrylamide gel. After electrophoresis, the proteins were electrophoretically transferred onto polyvinylidene difluoride membranes (Millipore), which were then stained with 0.5% Ponceau S to assure equal protein loading. The membranes were blocked with 5% powdered nonfat dry milk for 1 h in 25 mM Tris-HCl (pH 8.0), 144 mM NaCl, and 0.1% Tween 20 (TBS-T) and then incubated with an appropriate primary antibody followed by incubation with secondary antibody. After wash with TBS-T 3 times for 10 min each, the membranes were visualized using an enhanced chemiluminescent ECL assay kit (Santa Cruz Biotechnology) according to the manufacturer's recommended protocol.

Statistical analysis

All data are expressed as mean $\pm$ SD, and analyzed using ANOVA followed by least significant difference (LSD) *t*-test for post-hoc comparison. A P value of ≤ 0.05 was considered statistically significant.

Fig. 1. A scheme of the pressure-inducing apparatus. Cell dishes were placed in a steel-made chamber, and the chamber was sealed with a screw-lock lid with inlet and outlet ports. Compressed helium gas was released into the chamber to induce pressure through a long tube connected to the inlet port. The outlet port was connected to a sphygmomanometer and an air-release valve.

Fig. 2. Pressure did not change the proliferation and α -SMA mRNA expression of quiescent HSCs. Cultured HSCs for 12 hours were used as quiescent HSCs in this experiment. (A) Pressure from 0 to 80 mmHg was applied to quiescent HSCs for 1 or 24 h and the proliferation rate of the HSCs was assessed using a BrdU incorporation assay ($n=6$). (B) Pressure from 0 to 80 mmHg was applied to the HSCs for 1 h or 24 h and RT-PCR was performed for α -SMA and the products were resolved by agarose gel electrophoresis ($n=3$). Data are shown as mean $\pm$ SD. aHSC, activated HSC; qHSC, quiescent HSC. The number of observations (n) represents the number of independent cell preparations.

Fig. 3. Pressure induced a time and dose-dependent effect on activated HSC proliferation and on the expression of PCNA and type I collagen. Cultured HSCs for 14 days were used as activated HSCs in this experiment. (A) Pressure from 0 to 80 mmHg was applied to the HSCs for 1 or 24 h and the proliferation rate of the HSCs was assessed using a BrdU incorporation assay ($n=6$). (B-D) Pressure from 0 to 80 mmHg was applied to the HSCs for 1 h and immunoblotting study was carried out to examine the levels of type I collagen and PCNA. The signal intensity of type I collagen and PCNA bands was normalized to that of β actin ($n=4$). Data are shown as mean $\pm$ SD. *, $P<0.05$; **, $P<0.01$ compared with HSCs at 0 mmHg. The number of observations (n) represents the number of independent cell preparations.

Results

Pressure exerted a time and dose-dependent effect on activated HSC proliferation and expression of type I collagen and PCNA

An application of pressure from 0 to 80 mmHg for 1 h or 24 h did not change quiescent HSC (cultured for 12 hours) proliferation and expression of α -SMA mRNA (Fig. 2). Activated HSC (cultured for 14 days) proliferation was markedly increased by 1-h application of pressure from 10 to 20 mmHg (Fig. 3A). A higher pressure of 40–80 mmHg did not have noticeable effect. Pressure for 24 h did not affect the rate of activated HSC proliferation regardless of the pressure within the range of 0–80 mmHg. The expression of type I collagen and PCNA in activated HSCs was significantly increased in response to 1-h application of 10 or 20-mmHg pressure compared with the controls (0 mmHg) (Fig. 3B to 3D). However, the expression of these two proteins was markedly lowered in the activated HSCs subjected to pressure of 40 or 80 mmHg compared with the controls (0 mmHg) ($p < 0.05$ or 0.01) (Fig. 3C and 3D). These findings suggested that the effect of pressure on activated HSC proliferation and expression of PCNA and type I collagen was time- and dose-dependent. Based

on these results, activated HSCs were used for the following experiments.

Pressure caused dose-dependent changes in the mRNA levels of β_3 integrin

RT-PCR analysis showed that 1-h application of 5 to 20 mmHg significantly increased β_3 integrin mRNA compared with controls (0 mmHg) ($p < 0.01$) (Fig. 4A and 4D). Further increase of the applied pressure to 40 or 80 mmHg, however, significantly reduced the expression of β_3 integrin ($p < 0.05$ or 0.01). By contrast, 1-h application of 5 to 80 mmHg to the HSCs exerted no noticeable effects on the mRNA levels of PDGF receptor and TGF- β receptor 1 (Fig. 4A, 4B and 4C).

Pressure induced dose-dependent changes in the phosphorylation levels of Src, FAK, ERK1/2 and Akt

One-hour application of pressure of 10 or 20 mmHg induced significantly increased phosphorylation of Src, FAK, ERK1/2 and Akt ($p < 0.01$) (Fig. 5). Pressure beyond 40 mmHg resulted in reduced phosphorylation of these proteins, suggesting a dose-dependent effect of pressure on the phosphorylation of these signaling proteins in the HSCs.

Fig. 4. Pressure induced dose-dependent changes in the mRNA levels of β_3 integrin. Cultured HSCs for 14 days were used as activated HSCs in this experiment. Pressure from 0 to 80 mmHg was applied to the HSCs for 1 h and RT-PCR was performed for β_3 integrin, TGF- β receptor 1 and PDGF receptor and the products were resolved by agarose gel electrophoresis (A). The mRNA levels for these molecules were determined by normalizing against β actin mRNA level in the same sample (B-D) ($n=5$). Data are shown as mean $\pm$ SD. *, $P<0.05$; **, $P<0.01$ compared with HSCs at 0 mmHg. The number of observations (n) represents the number of independent cell preparations.

Fig. 5. Pressure induced the phosphorylation of Src, FAK, ERK1/2 and Akt. Cultured HSCs for 14 days were used as activated HSCs in this experiment. Pressure from 0 to 80 mmHg was applied to the HSCs for 1 h and immunoblotting assays using phospho-specific antibodies against Src, FAK, ERK1/2 and Akt were carried out (A). The relative phosphorylation levels of these proteins were determined by densitometric analysis (B to E) ($n=5$). Data are shown as mean $\pm$ SD. **, $P<0.01$ compared with HSCs at 0 mmHg. The number of observations (n) represents the number of independent cell preparations.

Inhibition of Src attenuated the effects of pressure on activated HSCs

Preincubation with herbimycin A markedly attenuated the phosphorylation of the downstream targets of Src,

FAK, Akt and ERK1/2 (Fig. 6), suggesting that the activation of these signaling proteins was Src-dependent. LY294002, a specific inhibitor of PI3K, markedly reduced the phosphorylation of Akt (Fig. 6A and 6C) but not the

Fig. 6. The phosphorylation of FAK, ERK1/2 and Akt was Src-dependent. Cultured HSCs for 14 days were used as activated HSCs in this experiment. Ten mmHg pressure was applied to the HSCs after 1 h treatment with herbimycin A (900 nM), LY294002 (25 μ M) or PD98059 (25 μ M). Immunoblotting assays using phospho-specific antibodies against Src, FAK, ERK1/2 and Akt were carried out (A). The relative phosphorylation levels of these proteins were determined by densitometric analysis (B to D) (n=4). Data are shown as mean $\pm$ SD. **, $P<0.01$ compared with HSCs at 0 mmHg without any drug treatment. The number of observations (n) represents the number of independent cell preparations.

Fig. 7. Pressure-induced increase in the proliferation rate and expression of PCNA and type I collagen was Akt-dependent. Cultured HSCs for 14 days were used as activated HSCs in this experiment. Ten mmHg pressure was applied to the HSCs after 1 h treatment with herbimycin A (900 nM), LY294002 (25 μ M) or PD98059 (25 μ M) and levels of PCNA and type I collagen was examined by immunoblotting assays (A). The relative levels of type I collagen and PCNA were determined by densitometric analysis (B, C) (n=5). The proliferation rate of HSCs was determined by a Brdu incorporation assay (D) (n=6). Data are shown as mean $\pm$ SD. **, $P<0.01$ compared with HSCs at 0 mmHg without any drug treatment. The number of observations (n) represents the number of independent cell preparations.

phosphorylation level of FAK and ERK1/2 (Fig. 6A to 6B and 6D).

Pressure application for 1 h increased the Brdu incorporation and expression of type I collagen and PCNA. Such an effect was markedly attenuated by herbimycin A and LY294002, and not by PD98059, a ERK1/2 inhibitor (Fig. 7), suggesting that pressure-induced changes in the proliferation rate and expression

of type I collagen and PCNA in the HSCs are Src-dependent and mediated through the PI3K-Akt pathway.

Discussion

Hydrostatic pressure participates in a variety of pathological processes such as cardiac enlargement [21]

and atherosclerosis [22] and is also likely involved in the pathogenesis of hepatic fibrosis [18]. Here, we show that pressure promotes the proliferation and increases protein levels of PCNA and type I collagen of the HSCs in a time- and dose-specific manner, suggesting possible involvement of pressure in the onset of hepatic fibrosis. PDGF and TGF- β are key growth factors in hepatic fibrosis and may be involved in the cellular response to pressure *in vitro* [23, 24]. The expression of PDGF receptor and TGF- β receptor 1 in the HSCs did not change in response to pressure, suggesting that the pressure-induced proliferation of HSCs and upregulation of PCNA and type I collagen were not mediated by the PDGF- or TGF- β -dependent signaling pathways.

Integrins play an essential role in mechanotransduction by linking the membrane to the cytoskeleton with their β -cytoplasmic domain. HSCs express several isoforms of integrins, especially $\alpha_3\beta_3$, which regulate the proliferation and apoptosis of HSCs by activating ERK1/2 MAPKs [25, 26]. In the present study, we found that 10-mmHg pressure applied over 1 h increased the mRNA levels of β_3 integrin, suggesting the involvement of integrin-mediated signaling pathways in the cellular response to pressure in the HSCs. FAK, a cytoplasmic tyrosine kinase, is an important downstream mediator involved in integrin-mediated signal transduction [27]. When FAK (Tyr397) is activated following integrin activation, it provides a high-affinity motif for Src SH2-binding [28]. The FAK/Src complex triggers the activation of downstream targets in the integrin-initiated signaling pathway [29]. Src also binds directly to the β_3 integrin tail and clustering of β_3 integrin activates Src [28]. We found that 10-mmHg pressure applied to the HSCs over 1 h induced a concurrent activation of FAK (Tyr397) and Src (Tyr418) via a Src-dependent mechanism. Our results demonstrate that Src, FAK and integrins interact with one another in the HSCs in response to pressure *in vitro*, implicating these signaling pathways in the pathogenesis of hepatic fibrosis.

The Src/FAK complex regulates downstream mediators such as Akt and ERK1/2, both of which are activated in response to pressure [30] and are important for HSC proliferation [16, 31]. We show here that 1-h application of 10-mmHg pressure to the HSCs induced the phosphorylation of Src and ERK1/2, which was attenuated by herbimycin A. These results suggest Src-dependent activation of the ERK1/2 signaling pathway in response to pressure in the HSCs. Other studies indicated increased cellular proliferation such as hypertrophy in

response to pressure was associated with FAK-dependent activation of the ERK1/2 signaling pathway [32]. However, in this study, we found that increased Brdu incorporation and expression of PCNA and type I collagen could be militated against by herbimycin A and LY294002 and not by PD98059, suggesting a central role of Akt-dependent mechanisms in the proliferation of HSCs in response to pressure. As the ERK1/2 signaling pathway plays critical roles in regulating diverse biological activities of HSCs such as apoptosis and migration [26, 33], the biological role of the activation of the ERK1/2 signaling pathway that we observed here requires further investigation.

In the present study, exposure to 10-mmHg pressure for 24 h did not induce proliferation of HSCs. Phosphorylation of both Src and FAK was increased by an application of 10-mmHg pressure for 10 min to 6 h, but was attenuated after 12 h to 24 h (data not shown), suggesting the decrease in Src and FAK phosphorylation partly contributed to the failure of 24-h application of 10-mmHg pressure to trigger the proliferation of HSCs although the mechanisms remain to be elucidated.

Moreover, no change was observed in Brdu incorporation or α -SMA mRNA expression of quiescent HSCs (cultured for 12 hours) exposed to the pressure from 0 to 80 mmHg, suggesting pressure did not affected the transdifferentiation of quiescent HSCs to fibroblasts. Taken together, our results indicate that dynamic change of interstitial fluid pressure could accelerate hepatic fibrosis, but only with under a condition that already activated HSCs.

In summary, we demonstrated here that pressure induces a time- and dose-dependent effect on cellular proliferation and the expression of cellular proteins important for cellular growth in the HSCs. In addition, pressure causes a dose-dependent activation of multiple cellular signaling pathways including the Src-dependent Akt signaling pathway, the FAK signaling pathway and the ERK1/2 pathways, suggesting the complexity of the cellular signaling response to pressure. Our findings highlighted a central role of Src-dependent signaling in the cellular response to pressure in the HSCs.

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References

- 1 Sato M, Suzuki S, Senoo H: Hepatic stellate cells: unique characteristics in cell biology and phenotype. *Cell Struct Funct* 2003;28:105-112.
- 2 Tsukada S, Parsons CJ, Rippe RA: Mechanisms of liver fibrosis. *Clin Chim Acta* 2006;364:33-60.
- 3 Friedman SL: Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008;88:125-172.
- 4 van Leeuwen DJ, Howe SC, Scheuer PJ, Sherlock S: Portal hypertension in chronic hepatitis: relationship to morphological changes. *Gut* 1990;31:339-343.
- 5 Blendis LM: Hepatocyte swelling and portal hypertension. *J Hepatol* 1992;15:4-5.
- 6 Abraldes JG, Iwakiri Y, Loureiro-Silva M, Haq O, Sessa WC, Groszmann RJ: Mild increases in portal pressure upregulate vascular endothelial growth factor and endothelial nitric oxide synthase in the intestinal microcirculatory bed, leading to a hyperdynamic state. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G980-987.
- 7 Perello A, Escorsell A, Bru C, Gilabert R, Moitinho E, Garcia-Pagan JC, Bosch J: Wedged hepatic venous pressure adequately reflects portal pressure in hepatitis C virus-related cirrhosis. *Hepatology* 1999;30:1393-1397.
- 8 Laine GA, Hall JT, Laine SH, Granger J: Transsinusoidal fluid dynamics in canine liver during venous hypertension. *Circulation Research* 1979;45:317-323.
- 9 Diresta GR, Nathan SS, Manoso MW, Casas-Ganem J, Wyatt C, Kubo T, Boland PJ, Athanasian EA, Miodownik J, Gorlick R, Healey JH: Cell proliferation of cultured human cancer cells are affected by the elevated tumor pressures that exist in vivo. *Ann Biomed Eng* 2005;33:1270-1280.
- 10 Watanabe S, Nagashio Y, Asaumi H, Nomiya Y, Taguchi M, Tashiro M, Kihara Y, Nakamura H, Otsuki M: Pressure activates rat pancreatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2004;287:G1175-1181.
- 11 Angele P, Yoo JU, Smith C, Mansour J, Jepsen KJ, Nerlich M, Johnstone B: Cyclic hydrostatic pressure enhances the chondrogenic phenotype of human mesenchymal progenitor cells differentiated in vitro. *J Orthop Res* 2003;21:451-457.
- 12 Huang H, Kamm RD, Lee RT: Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 2004;287:C1-11.
- 13 Rice DC, Dobrian AD, Schriver SD, Prewitt RL: Src autophosphorylation is an early event in pressure-mediated signaling pathways in isolated resistance arteries. *Hypertension* 2002;39:502-507.
- 14 Albinsson Hellstrand P: Integration of signal pathways for stretch-dependent growth and differentiation in vascular smooth muscle. *Am J Physiol Cell Physiol* 2007;293:C772-782.
- 15 Liu S, Calderwood DA, Ginsberg MH: Integrin cytoplasmic domain-binding proteins. *J Cell Sci* 2000;113:3563-3571.
- 16 Reif S, Lang A, Lindquist JN, Yata Y, Gabele E, Scanga A, Brenner DA, Rippe RA: The role of focal adhesion kinase-phosphatidylinositol 3-kinase-akt signaling in hepatic stellate cell proliferation and type I collagen expression. *J Biol Chem* 2003;278:8083-8090.
- 17 Guan JL: Focal adhesion kinase in integrin signaling. *Matrix Biol* 1997;16:195-200.
- 18 Okada Y, Tsuzuki Y, Hokari R, Miyazaki J, Matsuzaki K, Mataka N, Komoto S, Watanabe C, Kawaguchi A, Nagao S, Itoh K, Miura S: Pressure loading and ethanol exposure differentially modulate rat hepatic stellate cell activation. *J Cell Physiol* 2008;215:472-480.
- 19 De Minicis S, Seki E, Uchinami H, Kluwe J, Zhang Y, Brenner DA, Schwabe RF: Gene expression profiles during hepatic stellate cell activation in culture and in vivo. *Gastroenterology* 2007;132:1937-1946.
- 20 Oishi Y, Uezono Y, Yanagihara N, Izumi F, Nakamura T, Suzuki K: Transmural compression-induced proliferation and DNA synthesis through activation of a tyrosine kinase pathway in rat astrocytoma RCR-1 cells. *Brain Res* 1998;781:159-166.
- 21 Mori T, Chen YF, Feng JA, Hayashi T, Oparil S, Perry GJ: Volume overload results in exaggerated cardiac hypertrophy in the atrial natriuretic peptide knockout mouse. *Cardiovasc Res* 2004;61:771-779.
- 22 Roman MJ, Devereux RB, Kizer JR, Okin PM, Lee ET, Wang W, Umans JG, Calhoun D, Howard BV: High central pulse pressure is independently associated with adverse cardiovascular outcome the strong heart study. *J Am Coll Cardiol* 2009;54:1730-1734.
- 23 Uutela M, Wirzenius M, Paavonen K, Rajantie I, He Y, Karpanen T, Lohela M, Wiig H, Salven P, Pajusola K, Eriksson U, Alitalo K: PDGF-D induces macrophage recruitment, increased interstitial pressure, and blood vessel maturation during angiogenesis. *Blood* 2004;104:3198-3204.
- 24 Baker AB, Etnenson DS, Jonas M, Nugent MA, Iozzo RV, Edelman ER: Endothelial cells provide feedback control for vascular remodeling through a mechanosensitive autocrine TGF-beta signaling pathway. *Circ Res* 2008;103:289-297.
- 25 Patsenker E, Popov Y, Wiesner M, Goodman SL, Schuppan D: Pharmacological inhibition of the vitronectin receptor abrogates PDGF-BB-induced hepatic stellate cell migration and activation in vitro. *J Hepatol* 2007;46:878-887.
- 26 Zhou X, Murphy FR, Gehdu N, Zhang J, Iredale JP, Benyon RC: Engagement of alphavbeta3 integrin regulates proliferation and apoptosis of hepatic stellate cells. *J Biol Chem* 2004;279:23996-24006.
- 27 Saleem S, Li J, Yee SP, Fellows GF, Goodyer CG, Wang R: beta1 integrin/FAK/ERK signalling pathway is essential for human fetal islet cell differentiation and survival. *J Pathol* 2009;219:182-192.
- 28 Mitra SK, Schlaepfer DD: Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr Opin Cell Biol* 2006;18:516-523.
- 29 Cary LA, Guan JL: Focal adhesion kinase in integrin-mediated signaling. *Front Biosci* 1999;4:D102-113.
- 30 Franchini KG, Torsoni AS, Soares PH, Saad MJ: Early activation of the multicomponent signaling complex associated with focal adhesion kinase induced by pressure overload in the rat heart. *Circ Res* 2000;87:558-565.
- 31 Fan J, Shen H, Sun Y, Li P, Burczynski F, Namaka M, Gong Y: Bone morphogenetic protein 4 mediates bile duct ligation induced liver fibrosis through activation of Smad1 and ERK1/2 in rat hepatic stellate cells. *J Cell Physiol* 2006;207:499-505.
- 32 DiMichele LA, Doherty JT, Rojas M, Beggs HE, Reichardt LF, Mack CP, Taylor JM: Myocyte-restricted focal adhesion kinase deletion attenuates pressure overload-induced hypertrophy. *Circ Res* 2006;99:636-645.
- 33 Pasternack M Jr, Liu X, Goodman RA, Rannels DE: Regulated stimulation of epithelial cell DNA synthesis by fibroblast-derived mediators. *Am J Physiol* 1997;272:L619-630.