Stem Cell Research (2015) 14, 155–164

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

Cyclic mechanical stretching promotes migration but inhibits invasion of rat bone marrow stromal cells

Bingyu Zhang[a,1], Qing Luo[a,1], Zhe Chen[a], Jinghui Sun[a], Baiyao Xu[b], Yang Ju[b], Guanbin Song[a,*]

[a] Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, People's Republic of China
[b] Department of Mechanical Science and Engineering, Nagoya University, Nagoya 464-8603, Japan

Received 14 May 2014; received in revised form 9 January 2015; accepted 11 January 2015
Available online 17 January 2015

Abstract Bone marrow stromal cells (BMSCs, also broadly known as bone marrow-derived mesenchymal stem cells) are multipotent stem cells that have a self-renewal capacity and multilineage differentiation potential. Mechanical stretching plays a vital role in regulating the proliferation and differentiation of BMSCs. However, little is known about the effects of cyclic stretching on BMSC migration and invasion. In this study, using a custom-made cell-stretching device, we studied the effects of cyclic mechanical stretching on rat BMSC migration and invasion using a Transwell Boyden Chamber. The protein secretion of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) was detected by gelatin zymography, and the activation of focal adhesion kinase (FAK) and extracellular signal regulated kinase1/2 (ERK1/2) was measured by western blot. We found that cyclic mechanical stretching with 10% amplitude at 1 Hz frequency for 8 h promotes BMSC migration, but reduces BMSC invasion. FAK and ERK1/2 signals were activated in BMSCs after exposure to cyclic stretching. In the presence of the FAK phosphorylation blocker PF573228 or the ERK1/2 phosphorylation blocker PD98059, the cyclic-stretch-promoted migration of BMSCs was completely suppressed. On the other hand, cyclic mechanical stretching reduced the secretion of MMP-2 and MMP-9 in BMSCs, and PF573228 suppressed the cyclic-stretch-reduced secretion of MMP-2 and MMP-9. The decrease of BMSC invasion induced by mechanical stretching is partially restored by PF573228 but remained unaffected by PD98059. Taken together, these data show that cyclic mechanical stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway, but reduces BMSC invasion by decreasing secretion of MMP-2 and MMP-9 via FAK, independent of the ERK1/2 signal.

Introduction

Bone marrow stroma contains bone marrow stromal cells (BMSCs), also broadly known as bone marrow-derived

* Corresponding author at: College of Bioengineering, Chongqing University, Chongqing 400044, People's Republic of China. Fax: +86 23 65102507.
E-mail address: song@cqu.edu.cn (G. Song).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scr.2015.01.001
1873-5061/

mesenchymal stem cells. They are a subset of multipotent stem cells that can, given the appropriate conditions, differentiate into a variety of cell types, including osteoblasts, chondrocytes, and adipocytes (Salem and Thiemermann, 2010), suggesting a potential for BMSCs to promote tissue repair and regeneration in clinical practice (Hong et al., 2012). To better understand the characteristics of BMSCs and utilize them, recent extensive studies regarding BMSCs have focused on how chemical signals guide the physiological processes of BMSCs (Balakrishnan et al., 2010; Birmingham et al., 2012). Accumulating evidence has shown that mechanical factors, such as fluid shear stress, mechanical strain and the rigidity of the extracellular matrix, can regulate the proliferation and differentiation of BMSCs. It has been proven that both shear stress (Kim et al., 2014) and cyclic stretching (Kang et al., 2012; Kearney et al., 2010) could increase the expression of osteogenic markers. Cyclic stretching also serves as a driving factor for promoting the expression of genes related to smooth muscle cells (SMCs) in BMSCs (Hamilton et al., 2004; Park et al., 2004). Moreover, cyclic mechanical stretching could enhance rat BMSC proliferation (Yuan et al., 2012a).

BMSCs are multipotent progenitor cells in the bone marrow stroma. It is reported that a subset of BMSCs are pericytes, found on the abluminal side of marrow sinusoids (Sacchetti et al., 2007). By being pericytes of marrow sinusoids, BMSCs are inevitably subjected to the hemodynamic forces generated from the blood pressure and blood flow in the form of cyclic stretching and shear stress (Haga et al., 2007). BMSC migration is necessary for them to move into a resorption site to begin to regenerate bone. Therefore, to facilitate the efficiency of BMSC transplantation and regulate their motility, it is worthwhile to investigate the influence of hemodynamic forces on the motility of BMSCs. Our previous works have illustrated the effect of shear stress on the migration of BMSCs (Yuan et al., 2012b), while the influence of cyclic mechanical stretching on BMSC migration and invasion remains unclarified.

Cell motility is a complex physicochemical process that leads to the translocation of the cell body across two-dimensional (2D) surfaces, through basement membranes, or through three-dimensional (3D) interstitial tissues (Ridley et al., 2003). During cell movement through basement membranes or 3D tissues, two essential properties are required: the generation of motile forces and the production of matrix-degrading proteinases that enable cells to penetrate extracellular cell matrix (ECM) hindrance. Matrix metalloproteinases (MMPs) are a family of neutral endopeptidases that participate in the degradation of the ECM components. Previous studies have shown that mechanical stimuli affect the secretion of MMPs in a variety of cell types, such as endothelial cells, smooth muscle cells, and periodontal ligament cells (Garanich et al., 2005; Yamane et al., 2010; Zheng et al., 2012).

Focal adhesion kinase (FAK) is a focal adhesion-associated protein kinase in the focal adhesion site that composes part of mechanotransduction in cells in response to mechanical stimulation (Li et al., 1997). Moreover, FAK plays an important role in cellular invasion and migration. FAK-deficient cells show reduced cell motility and enhanced focal adhesion contact formation compared to control cells (Ilić et al., 1995). By contrast, FAK expression is elevated in human cancers where cell invasion through matrix and tissue barriers requires increased cell motility (Lim et al., 2008). Extracellular signal-regulated kinase1/2 (ERK1/2) is a well-known downstream signalling molecular of FAK and is a force-activated protein kinase. It can rapidly be activated by shear stress and cyclic stretching (Li et al., 1997; Yuan et al., 2012a). Activated ERK1/2 regulates the activity of MMPs, leading to the degradation of ECM and enhanced cell motility (Babykutty et al., 2012; Tan et al., 2008).

Given that migration and invasion are the two crucial characteristics that enable cell motility, in this study, we aim to investigate the effects of cyclic mechanical stretching on migration and invasion of rat BMSCs and the possible functional role of the FAK-ERK 1/2 signalling pathway and of MMP-2 and MMP-9 in stretch-induced changes in BMSC motility.

Materials and methods

Cell isolation and characterisation

The femurs and tibias from two-month-old male Sprague–Dawley rats (Laboratory Animal Centre, Third Military Medical University, China) were sawn open, and the gelatinous bone marrow was extracted under sterile conditions. All of the procedures were approved by the Chongqing Science and Technology Commission. Rat BMSCs were obtained by density gradient centrifugation with 1.073 g/mL Percol (Sigma-Aldrich, Saint Louis, MO) at 2500 rpm/min for 30 min and were then cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS, Hyclone, Logan, UT), 100 U/mL penicillin, and 100 μg/mL streptomycin in a standard incubator under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture medium was replaced every three days. After reaching confluence, the cells were subcultured with 0.25% trypsin-0.02% EDTA at a density of 2.5×10^3 cells per cm^2. Cells from passages 2–5 were used for the experiments.

The isolated cells were analysed by flow cytometry. Briefly, the cells were collected and incubated with antibodies according to the manufacturer's protocols. A FITC-conjugated mouse anti-rat CD11b/c antibody, FITC-conjugated hamster anti-rat CD29 antibody, PE-conjugated mouse anti-rat CD45 antibody, PE-conjugated mouse anti-rat CD54 antibody and the corresponding isotype antibodies were purchased from Biolegend (CA, USA). After being washed twice with PBS, the cells were resuspended in 500 μL of wash buffer and analysed by flow cytometer (BD, Oxford, UK).

Application of cyclic stretching

We employed a mechanical stretching device (Model ST-140, STREX Co., Ltd, Osaka, Japan) to generate a stretching loading on cultured cells in vitro. Briefly, the device consists of a control unit, a strain unit, and rectangular, elastic silicone chambers (Fig. 1a). During the stretching experiments, only the strain unit was put into the incubator (Fig. 1b). The chambers were designed for use in the strain unit, which was driven by an eccentric motor that allowed variations in the magnitude (0–20%) and frequency (0–1.5 Hz) of the applied strain. This automated instrument was designed to hold four or five chambers with precise uniaxial mechanical strain applied synchronously. The stretching over the entire cell culture surface was uniform.

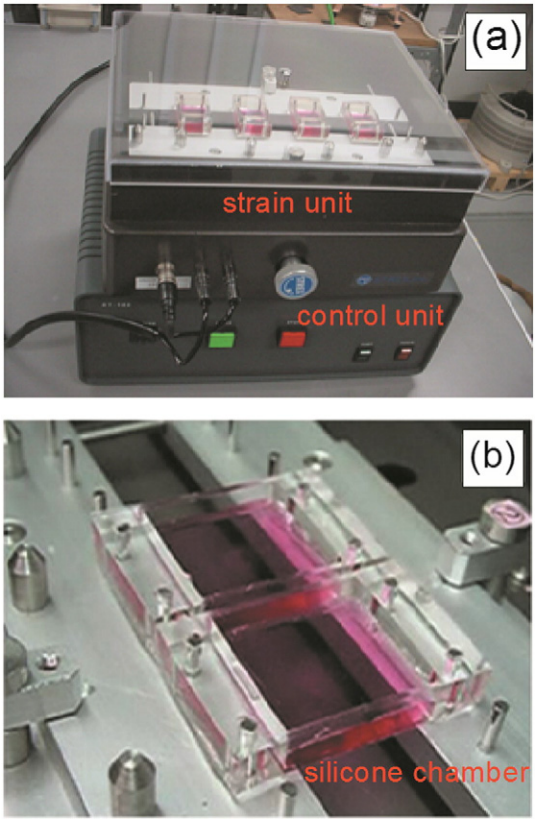

Figure 1 Mechanical stretching device for the application of strain (Model ST-140, Osaka, Japan). The device consists of a control unit, a strain unit, and elastic silicone chambers. Cells are seeded on a substrate in the chamber, and the chamber is mounted on the strain unit using four holes and hooks. This device is designed to hold four or five chambers, with a precise uniaxial mechanical strain applied synchronously. During the stretching experiments, only the strain unit is put into the incubator. (a) Photograph of STREX cell stretching system. (b) Magnified image of the strain unit.

The cells were seeded at a density of 1×10^4 cells/cm^2 into a chamber precoated with 5 μg/mL rat tail type I collagen (Shengyou biotechnology Co., Hangzhou, China) and allowed to nearly reach confluence after about two days of cultivation. After synchronization in DMEM without FBS for 12 h, the chamber was then mounted on the strain unit of the strain instrument using four holes and hooks. The cells in the silicone chamber were exposed to stretching treatment at 1.0 Hz, 10% strain for 8 h. As controls, static cells were cultured in a chamber under the same conditions, but were not subjected to any strain.

Cell migration assay

Cell migration was assessed using a Transwell Boyden Chamber (pore diameter: 8 μm. Millipore, Billerica, MA). In brief, after exposure to stretching, BMSCs were harvested and counted, and approximately 3×10^4 cells were resuspended in 150 μL of medium with 1% FBS and were added into the upper compartment of the chamber. The lower compartment was filled with 700 μL of medium with 2% FBS as a chemoattractant. The cells were allowed to migrate at 37 °C in an atmosphere of 95% air and 5% CO_2 for 12 h. A cotton-tipped swab was used to remove the cells on the upper side of the filters, and the cells that migrated through the pores were strained with 0.05% crystal violet in PBS for 30 min. Then, the filters were washed with PBS. The BMSCs on the underside of the filters were counted under a microscope. Images were taken with four fields selected randomly from each insert. The number of cells in each field was counted and averaged. Migration is expressed as fold change over the control cells (cultured in the same conditions without any stretching).

Cell invasion assay

The invasion assay was performed using the Transwell Boyden Chamber coated with Matrigel (Becton Dickinson Labware, Bedford, MA). Briefly, Matrigel was mixed with coating buffer and transferred into the insert of the chamber. After insert coating was finished, stretched cells (approximately 6×10^4 cells in 150 μL medium with 1% FBS) were added into the insert and the insert was placed into a 24-well plate containing 700 μL of medium with 10% FBS as a chemoattractant. After 48 h of incubation at 37 °C, the inner surfaces of the filters were swabbed to remove non-invading cells, then the invaded cells were stained with 0.05% crystal violet in PBS for 30 min. BMSCs on the underside of filters were counted under a microscope. Images were taken with four randomly selected fields for each insert. The number of cells in each field was counted and averaged. Invasion is expressed as fold change over the control cells (cultured in the same conditions without any stretching).

MTT assay

An MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) assay was performed to evaluate the effect of stretching on the proliferation of BMSCs. After being exposed to stretching, the cells were cultured for 0 h, 12 h and 48 h, and then, MTT reagent (5 mg/mL, Sigma-Aldrich, Saint Louis, MO, USA) was added into the chamber and incubated at 37 °C for 4 h. The media were removed and the formazan crystals in the cells were dissolved with dimethyl sulfoxide (DMSO). The formazan was then quantified using a microplate reader (Model 680, Bio-Rad, Hercules, CA, USA). For each time point, the control cells were not stretched but were grown in the same vessel and culture medium. The percentage of cell proliferation was determined by normalizing the optical density (OD) value of the stretched group to that of the corresponding control group.

Gelatin zymography

To assess the secretion of MMP-2 and MMP-9, gelatin substrate zymography was used. Briefly, after BMSCs were exposed to mechanical stretching, the supernates from control cells (cultured in the same conditions without any stretching) and stretched cells were collected and then subjected to electrophoresis using SDS-polyacrylamide gels co-polymerized with gelatine. The protein concentrations of the supernates were determined by the BCA protein

quantitation kit (KeyGEN Biotech, Nanjing, China), and equal amounts of proteins of each group were applied to the gel. The gels were renatured in a 2.5% TritonX-100 solution and then incubated overnight at 37 °C in a developing buffer (Tris 0.5 M, Brij35 0.2%, NaCl 2 M, $CaCl_2$ 50 mM, pH7.6). Then, the gels were consecutively stained with Coomassie brilliant blue and destained to let the bands appear. A semiquantitative evaluation of the bands was performed by densitometry (GS-800, Bio-Rad, Hercules, CA, USA).

SDS-polyacrylamide gel electrophoresis and western blot analysis

BMSCs were exposed to a stretching treatment with 1.0 Hz, 10% strain for 0, 5, 15, 30, or 60 min, then were washed with ice-cold PBS and digested immediately. The proteins were extracted using a cell lysis buffer. After electrophoretic separation by 8% SDS-polyacrylamide gel electrophoresis, the proteins were electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were then blocked with Tris-buffered saline containing 0.1% Tween-20 (TBST) and 5% skim milk for 1 h at room temperature. A FAK rabbit Ab, phospho-FAK (p-FAK) rabbit Ab, ERK1/2 rabbit mAb, phospho-ERK1/2 (p-ERK1/2) rabbit mAb (Cell Signaling Technology, Danvers, MA, USA) and β-actin rabbit mAb (4A Biotech, Beijing, China) were used according to the manufacturers' protocols, and the membranes were incubated overnight with these antibodies at 4 °C with slight shaking. Thereafter, the membranes were washed three times in TBST and further incubated with an HRP-conjugated antibody (goat anti-rabbit IgG, Sigma-Aldrich Saint Louis, MO, USA) for 1 h at room temperature. Finally, the membranes were washed three times in TBST, and the signals were developed using an enhanced chemiluminescence kit (KeyGEN Biotech, Nanjing, China). A semiquantitative evaluation of the bands was performed by densitometry (VersaDoc, Bio-Rad, Hercules, CA, USA). The levels of FAK, p-FAK, ERK1/2 and p-ERK1/2 proteins were determined through their normalization to the protein level of β-actin.

For inhibition of FAK and ERK activation, dissociated cells were incubated with PF573228 (10 μM, Sigma-Aldrich, Saint Louis, MO, USA) or PD98059 (50 μM, Sigma-Aldrich, Saint Louis, MO, USA) for 30 min at 37 °C before they were subjected to cyclic mechanical stretching.

Statistical analysis

The data are expressed as the means ± SD, and the results were analysed statistically using Student's *t* test and analysis of variance. Bonferroni post-hoc tests were used when the *p* value indicated a significant difference between the groups, and $p < 0.05$ was deemed to be statistically significant.

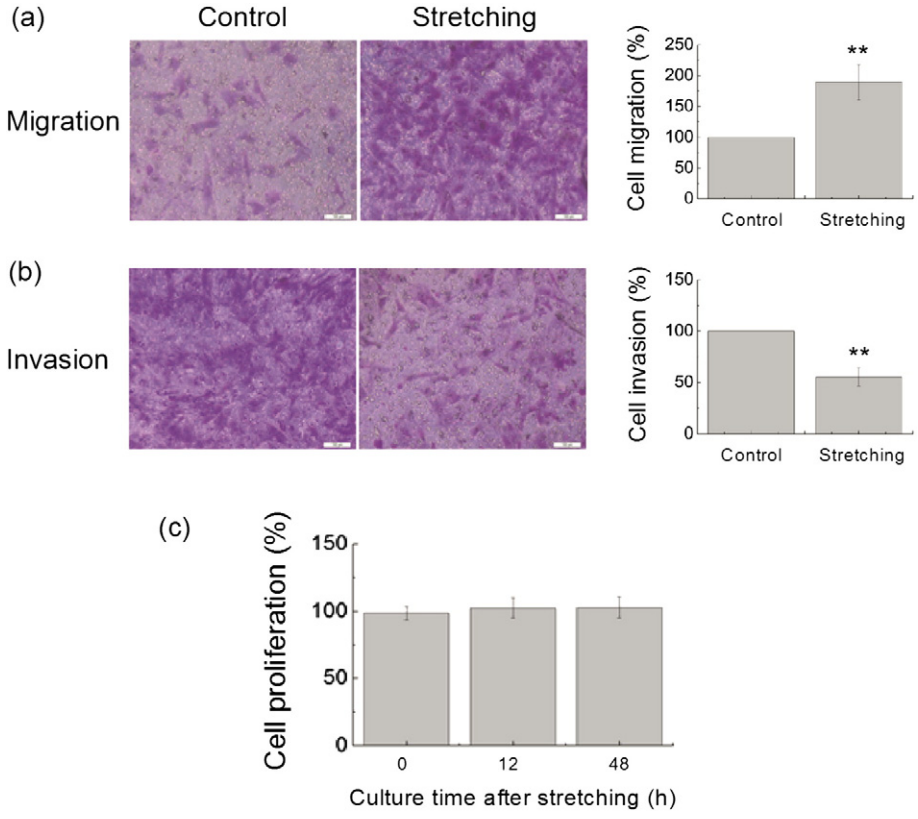

Figure 2 Cyclic stretching promotes migration and reduces invasion of BMSCs. The cells were exposed to cyclic stretching with 10% amplitude at 1 Hz frequency for 8 h, and then, cell migration (a) and invasion (b) were detected using a Transwell Boyden Chamber. Bars = 100 μm. (c) Effects of cyclic stretching on the proliferation of BMSCs (MTT assay). Data are expressed as the means ± SD; $n = 4$. $^{**}p < 0.01$ compared with control group (cultured in the same conditions without any stretching).

Results

Cell characterisation

The cultured cells showed an elongated, spindle shape and fibroblast-like morphology. The flow cytometry data showed that 99.2% of the cells expressed CD29 and 99.1% expressed CD54, while less than 1% of the cell population expressed CD45 and CD11b/c (Fig. S1a). These characteristics are consistent with observations regarding rat BMSCs from previous reports (Harting et al., 2008; Ocarino et al., 2008).

Cyclic stretching promotes migration but reduces invasion of BMSCs

BMSCs were exposed to cyclic stretching with 10% amplitude at 1 Hz for 8 h, and the migration and invasion of BMSCs were then detected using a Transwell Boyden Chamber. As shown in Fig. 2, cyclic stretching significantly promoted migration, but decreased invasion of BMSCs (Figs. 2a and b). To determine whether cell proliferation affected the migration and invasion of BMSCs, the cells were cultured for 0, 12 or 48 h after stretching and an MTT assay was performed. The results showed that the proliferation of stretched BMSCs was not obviously changed within 48 h when compared with that of the control group, which was not stretched but grown in the same vessel and medium (Fig. 2c), indicating that cyclic stretching influences the migration and invasion of BMSCs independent of the change in cell number. To confirm whether the cyclic stretching drove a differentiation of BMSCs, cell characterization was performed after 8 h of stretching plus 48 h of static culture. The results showed that the cells still exhibited an elongated, spindle shape and fibroblast-like morphology and that 99.5% of the cells expressed CD29, 98.9% of the cells expressed CD54, 0.1% of the cells expressed CD11b/c and 0.7% of the cells expressed CD45 (Fig. S1b), which are classical characteristics of BMSCs and are similar to the characteristics of cells before being subjected to stretching. These results suggest that the phenotype of BMSCs was not changed by stretching.

Cyclic stretching increases FAK and ERK1/2 phosphorylation in BMSCs

Activation of the FAK and ERK1/2 signalling pathways is of importance for cell motility. To determine whether stretching

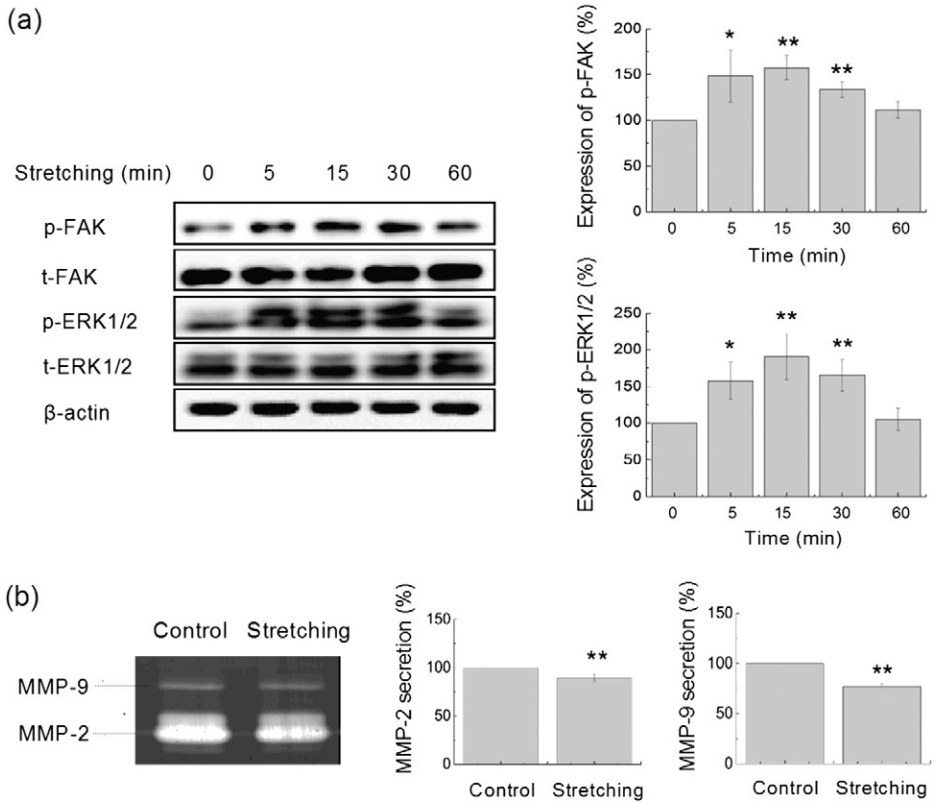

Figure 3 Effects of cyclic stretching on activation of the FAK- ERK1/2 pathway and secretions of MMP-2 and MMP-9 in BMSCs. (a) BMSCs were exposed at 10% strain at 1 Hz for 0, 5, 15, 30, or 60 min, and the protein expression levels of total FAK (t-FAK), phosphorylation of FAK (p-FAK) and total ERK1/2 (t-ERK1/2), phosphorylation of ERK1/2 (p-ERK1/2) were analysed by western blot. The results of the densitometric analysis of FAK and ERK1/2 activation were normalized to the levels of β-actin. Data are expressed as the means ± SD; $n = 3$; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control (0 min). (b) Gelatine zymograms showed that cyclic stretching reduced secretion of MMP-2 and MMP-9. Data are expressed as the means ± SD; n = 4; $^{**}p < 0.01$ compared with control (cultured in the same conditions without any stretching).

could activate the FAK and ERK1/2 signalling pathways, which might contribute to stretch-induced BMSC migration and invasion, BMSCs were exposed to cyclic stretching with 10% amplitude at 1 Hz for 0, 5, 15, 30, or 60 min, and expression of p-FAK and p-ERK1/2 was detected by western blot. We found that both p-FAK expression and p-ERK1/2 expression increased rapidly after stretching and peaked at 15 min and then gradually fell back to the control level at 60 min (Fig. 3a). These results demonstrated that cyclic stretching stimulation could activate the FAK and ERK1/2 signals in BMSCs.

Cyclic stretching reduces secretion of MMP-2 and MMP-9 in BMSCs

MMPs play an important role in cell invasion. To study whether cyclic stretching alters expression of MMP-2 and MMP-9 in BMSCs, gelatine zymography was employed to examine the secretion of MMP-2 and MMP-9. The result showed that a significant decrease of the MMP-2 and MMP-9 secretions was observed in BMSCs treated with cyclic stretching (Fig. 3b).

Cyclic stretching reduces BMSC invasion by decreasing the secretion of MMP-2 and MMP-9 via a FAK signal, but not an ERK1/2 signal

To identify the relationship between the activation of the FAK-ERK1/2 signalling pathway and stretch-changed BMSC migration and invasion, FAK and ERK1/2 inhibitors were used to determine the roles of FAK and ERK1/2 signals, respectively. The FAK inhibitor PF573228 at 10 μM suppressed the elevation of the p-FAK and p-ERK1/2 expression induced by

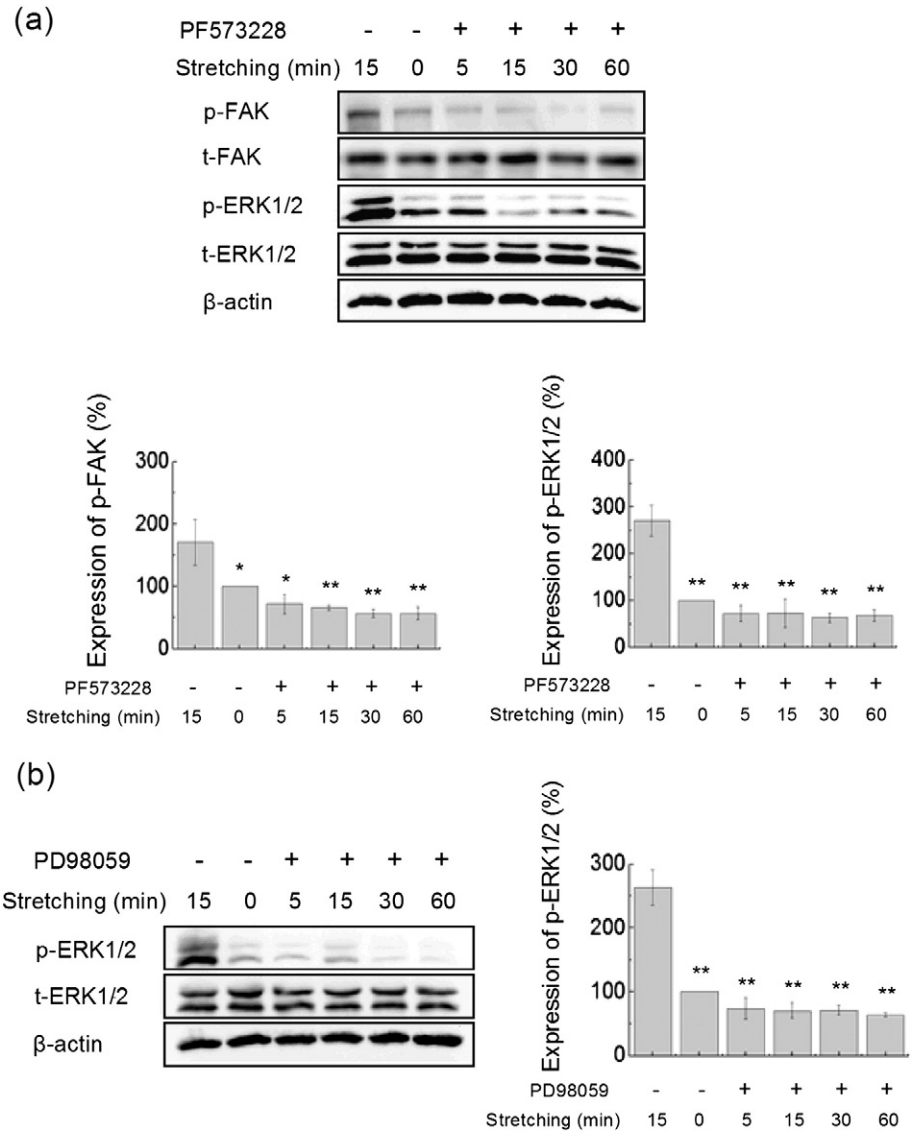

Figure 4 Effects of the FAK or ERK1/2 inhibitor on the activation of FAK or ERK1/2 in BMSCs. (a) The FAK inhibitor PF573228 (10 μM) suppressed the elevation of p-FAK and p-ERK1/2 activation induced by cyclic stretching. (b) The ERK1/2 inhibitor PD98059 (50 μM) suppressed the elevation of p-ERK1/2 activation induced by cyclic stretching. Data are expressed as the means ± SD; n = 3; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control (stretching for 15 min without inhibitor).

cyclic stretching at each time point (Fig. 4a). Furthermore, ERK1/2 inhibitor PD98059 at 50 μM suppressed the elevation of the p-ERK1/2 expression induced by cyclic stretching at each time point (Fig. 4b).

To understand the possible relationship between the FAK-ERK1/2 pathway and the secretion of MMP-2 and MMP-9, we investigated MMP-2 and MMP-9 secretions in stretched BMSCs in the presence of the FAK or ERK1/2 inhibitor. Gelatine zymograms showed that the FAK inhibitor PF573228 rescued the stretch-reduced secretion of MMP-2 and MMP-9; however, the ERK1/2 inhibitor PD98059 had no significant effect on the stretch-reduced secretion of MMP-2 and MMP-9 (Fig. 5). Furthermore, PF573228 also resumed the cyclic-stretch-reduced BMSC invasion to some extent, despite the fact that no statistical difference was observed (Figs. 6a and b). BMSC invasion alterations were not seen in stretched cells treated with PD98059 (Figs. 6a and b). These data indicate that cyclic stretching reduces BMSC invasion by decreasing the secretion of MMP-2 and MMP-9 via a FAK signal, but not an ERK1/2 signal.

Cyclic stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway

Transwell chamber assays further demonstrated that the blockade of the FAK or ERK1/2 signal inhibited the migration of BMSCs facilitated by cyclic stretching (Figs. 6a and c). These results confirmed that cyclic stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway.

Discussion

In the present work, we demonstrated that cyclic mechanical stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway, but reduces BMSC invasion by decreasing secretion of MMP-2 and MMP-9 via activation of the FAK signal, independent of the ERK1/2 signal. These data indicate that mechanical stretching is an important regulatory factor for BMSC motility.

Migration and invasion are the two crucial characteristics of cell mobility. Migration is often used to describe the movement of the cells themselves and has usually been studied on 2D surfaces. The cytoskeleton network is hypothesized to play a major role in determining the mechanical properties of living cells during cell migration (Stricker et al., 2010). Cell invasion is generally used to describe the tumour process, which focuses on the penetration of cells into ECM hindrance. MMPs are a family of neutral endopeptidases and participate in the degradation ECM components in cell invasion (Newby, 2006). Some studies reported that mechanical or chemical stimulation consistently affects the abilities of cell migration and invasion (Palumbo et al., 2000; Yang et al., 2014). Interestingly, in our study, we found that cyclic stretching promotes BMSC migration and reduces BMSC invasion. This finding suggests that mechanical stretching could differently regulate the migration and invasion abilities of BMSCs.

In our study, the FAK-ERK1/2 signalling pathway was activated in BMSCs after exposure to cyclic stretching. PF573228 or PD98059 inhibited stretch-induced phosphorylation of FAK and ERK1/2 and blocked stretch-promoted BMSC migration, which provides the first demonstration of the correlation between the FAK-ERK1/2 pathway and stretch-promoted BMSC migration. However, cyclic mechanical stretching reduces BMSC invasion by decreasing the secretion of MMP-2 and MMP-9 via activation of the FAK signal, independent of the ERK1/2 signal. In agreement with our results, Hsia et al. demonstrated that FAK differentially regulates cell migration and invasion in different pathways (Hsia et al., 2003).

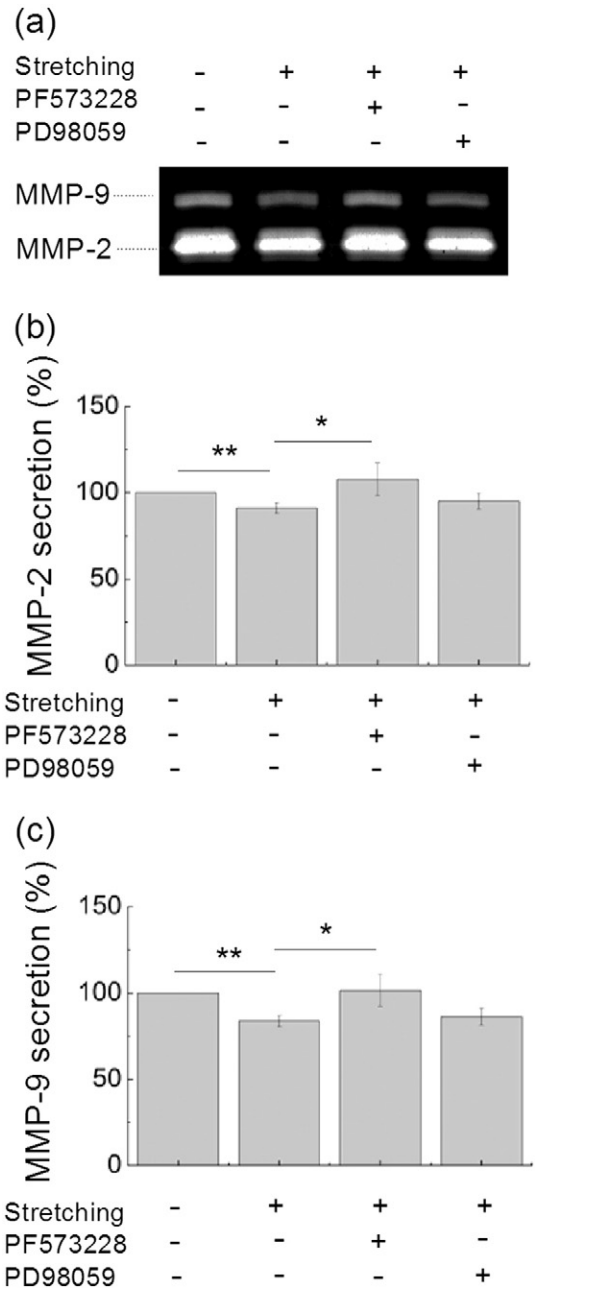

Figure 5 Effects of the FAK or ERK1/2 inhibitor on the secretion of MMP-2 and MMP-9 induced by cyclic stretching. (a) FAK inhibitor PF573228 (10 μM) rescued the stretch-reduced secretion of MMP-2 and MMP-9, but the ERK1/2 inhibitor PD98059 (50 μM) had no significant effect on stretch-reduced secretion of MMP-2 and MMP-9. (b–c) Quantification of secretion of MMP-2 and MMP-9. Data are expressed as the means ± SD; $n = 4$; $^{*}p < 0.05$ and $^{**}p < 0.01$.

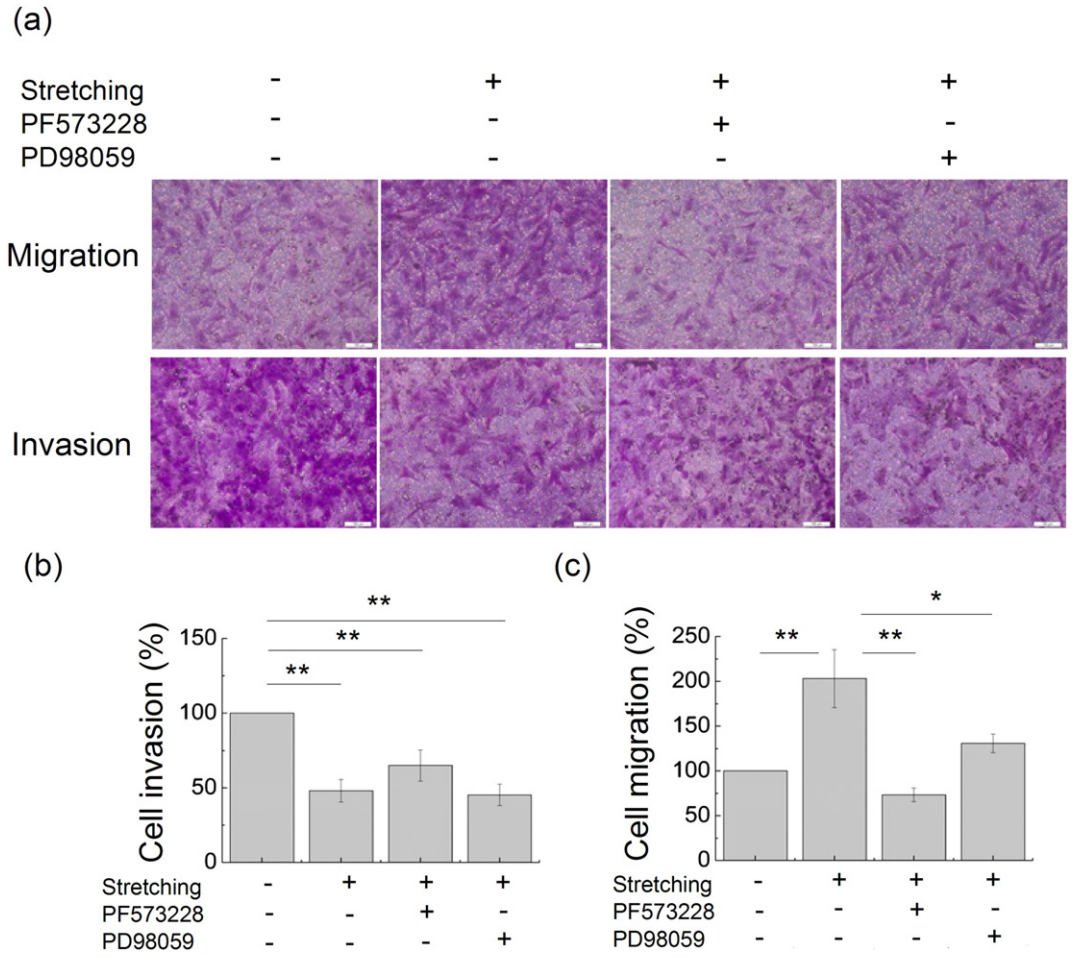

Figure 6 Effects of FAK or ERK1/2 inhibitor on the cyclic stretch-changed invasion and migration of BMSCs. (a) A representative result of migration and invasion of BMSCs. Bars = 100 μm. (b) Quantitation of BMSC invasion. PF573228 resumed stretch-reduced BMSC invasion to some extent (no significant difference). The ERK1/2 inhibitor PD98059 (50 μM) had no significant effect on stretch-reduced BMSC invasion. (c) Quantitation of BMSC migration. Pretreatment with the FAK inhibitor PF573228 (10 μM) or ERK1/2 inhibitor PD98059 (50 μM) for 30 min blocked the increase of BMSC migration induced by cyclic stretching. Data are expressed as the means ± SD; $n = 4$; $^{*}p < 0.05$ and $^{**}p < 0.01$.

It is generally believed that FAK signalling plays a pivotal role in invasion, migration and MMP production of cells (Natarajan et al., 2003). ERK1/2 is a well-known downstream effector of FAK. Some studies have shown that upregulation of FAK phosphorylation promotes MMP-2 and MMP-9 secretions through the ERK1/2 signal (Howe et al., 2002; Li et al., 2013). The activation of ERK1/2 regulates the activity of MMPs, leading to the degradation of ECM and cell invasion or migration (Babykutty et al., 2012; Chen et al., 2012; Tan et al., 2008). In our studies, the cyclic stretch-induced activation of FAK reduced the secretion of MMP-2 and MMP-9, but the ERK1/2 signal did not participate in this activation. It is suggested that other downstream signalling molecules of FAK are involved in the regulation of cyclic stretch-decreased MMP-2 and MMP-9 secretions. Several FAK downstream molecules have been proven to participate in the modulation of MMP-2 and MMP-9 expression, such as RhoA (Li et al., 2008), JNK1/2 (Lin and Shih, 2014), and Rac 1 (Park and Jeon, 2012). Moreover, these molecules can regulate cell migration or invasion (Chen et al., 2002; Lin and Shih, 2014; Park and Jeon, 2012). Thus, we speculate that these signalling molecules may be involved in the regulation of MMP-2 and MMP-9. However, more research is needed to confirm this. Our experiments indicate that cyclic stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway, but reduces BMSC invasion by decreasing the secretion of MMP-2 and MMP-9 via the activation of the FAK signal, independent of the ERK1/2 signal. These results will help us to better understand how the different mechanisms of migration and invasion of BMSCs are influenced by cyclic stretching.

In vivo, the stiffness and density of the surrounding ECM present an additional physical challenge to the moving cell body. Two principal mechanisms are known for moving cells to overcome these constraints: (i) proteolytic ECM degradation leading to gap widening and cell-generated trail formation and (ii) elastic and plastic deformations of the cell body to fit through the available space. If a cell is unable to "squeeze" through a particularly narrow region, it employs a third mechanism to maintain migration, i.e., retraction of already established protrusions and repolarization to explore the adjacent environment for an alternative route, thereby

bypassing the obstacle (Friedl et al., 2011). Therefore, ECM deposition is very important for cell invasion. MMPs are a family of neutral endopeptidases and participate in the degradation of ECM components. Among them, MMP-2 and MMP-9 have received considerable attention, as high levels of these MMPs are correlated with ECM deposition (De Mello Malheiro et al., 2009). In human prostate cancer, enhanced MMP-2 and MMP-9 expression contributed to tumour invasion and metastasis (Aalinkeel et al., 2011; Reis et al., 2011). Palumbo et al. reported that shear stress reduces MMP-2 expression, which is associated with the inhibition of smooth muscle cell invasion and migration (Palumbo et al., 2000). Interestingly, in our study, the FAK phosphorylation inhibitor PF573228 rescued mechanical stretch-reduced MMP-2 and MMP-9 secretions; however, stretch-reduced BMSC invasion was not obviously restored. Two possibilities might explain this. First, during the process of cell invasion, efficient invasion not only depends on the movement ability of the cells themselves but also on their ability to degrade the ECM. Thus, taken from the findings in our study, we speculate that due to the decreased migration ability induced by the FAK inhibitor, the stretch-reduced BMSC invasion could not be fully restored, even if the inhibitor blocked stretch-decreased MMP-2 and MMP-9 secretions. Another more likely possibility is that some other mechanisms may play important roles in cyclic-stretch-decreased cell invasion.

In summary, our findings demonstrate that cyclic mechanical stretching promotes the migratory potential of BMSCs via the FAK-ERK1/2 signalling pathway, but reduces BMSC invasion by decreasing the secretion of MMP-2 and MMP-9 via the FAK signal. To our knowledge, this is the first study to explore the effects of mechanical stretching on BMSC migration and invasion and their related mechanisms. A proposed schematic diagram of the mechanical stretching regulation of BMSC motility is shown in Fig. 7. BMSC motility is a complex process affected by multiple mechanical and chemical factors and their coupling. Our data identify the FAK-ERK1/2 signalling pathway as a critical mediator of BMSC motility induced by mechanical stretching. These results may be advantageous for a better understanding of mechanotransduction in BMSCs and for further studies of mechanochemical regulation in BMSC movement in basic and clinical research.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scr.2015.01.001.

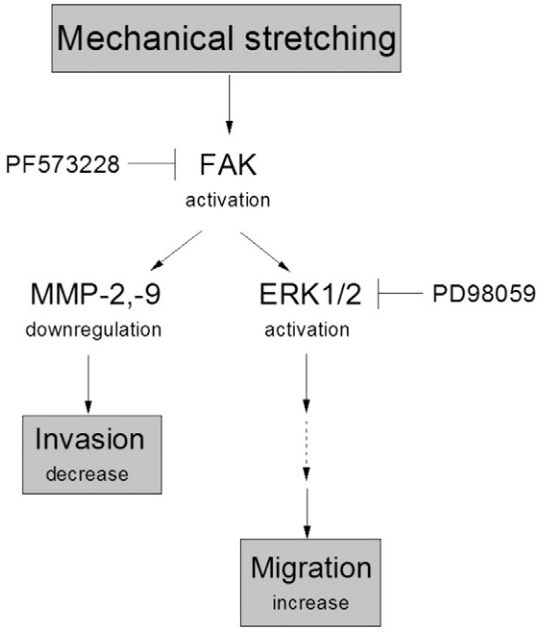

Figure 7 Proposed schematic diagram of regulation in BMSC motility by mechanical stretching. Cyclic mechanical stretching promotes BMSC migration via the FAK-ERK1/2 signalling pathway, which could be blocked by FAK inhibitor PF573228 or ERK1/2 inhibitor PD98059. Cyclic stretching reduces BMSC invasion by decreasing secretion of MMP-2 and MMP-9 through the activation of FAK but independent of the ERK1/2 signal.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Nos. 11032012, 11102240, and 11272365), the 111 Project (No. 06023), the Visiting Scholar Foundation of Key Laboratory of Biorheological Science and Technology (Chongqing University), the Ministry of Education of China (No. CQKLBST-2012-008), and the Research Fund for the Doctoral Program of Higher Education of China (No. 20130191110029).

References

Aalinkeel, R., Nair, B.B., Reynolds, J.L., Sykes, D.E., Mahajan, S.D., Chadha, K.C., Schwartz, S.A., 2011. Overexpression of MMP-9 contributes to invasiveness of prostate cancer cell line LNCaP. Immunol. Investig. 40 (5), 447–464.

Babykutty, S., Suboj, P., Srinivas, P., Nair, A.S., Chandramohan, K., Gopala, S., 2012. Insidious role of nitric oxide in migration/invasion of colon cancer cells by upregulating MMP-2/9 via activation of cGMP–PKG–ERK signaling pathways. Clin. Exp. Metastasis 29 (5), 471–492.

Balakrishnan, K., Burger, J.A., Quiroga, M.P., Henneberg, M., Ayres, M.L., Wierda, W.G., Gandhi, V., 2010. Influence of bone marrow stromal microenvironment on forodesine-induced responses in CLL primary cells. Blood 116 (7), 1083–1091.

Birmingham, E., Niebur, G.L., McHugh, P.E., Shaw, G., Barry, F.P., McNamara, L.M., 2012. Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. Eur. Cell Mater. 23, 13–27.

Chen, B.H., Tzen, J.T., Bresnick, A.R., Chen, H.C., 2002. Roles of Rho-associated kinase and myosin light chain kinase in morphological and migratory defects of focal adhesion kinase-null cells. J. Biol. Chem. 277 (37), 33857–33863.

Chen, Y.Y., Liu, F.C., Chou, P.Y., Chien, Y.C., Chang, W.S., Huang, G.J., Wu, C.H., Sheu, M.J., 2012. Ethanol extracts of fruiting bodies of *Antrodia cinnamomea* suppress CL1-5 human lung adenocarcinoma cells migration by inhibiting matrix metalloproteinase-2/9 through ERK, JNK, p38, and PI3K/Akt signaling pathways. Evid. Based Complement. Alternat. Med. 2012, 378415.

De Mello Malheiro, O.C., Giacomini, C.T., Justulin, L.A. Jr, Delella, F.K., Dal-Pai-Silva, M., Felisbino, S.L., 2009. Calcaneal tendon regions exhibit different MMP-2 activation after vertical jumping and treadmill running. Anat. Rec. (Hoboken) 292 (10), 1656–1662.

Friedl, P., Wolf, K., Lammerding, J., 2011. Nuclear mechanics during cell migration. Curr. Opin. Cell Biol. 23 (2), 55–64.

Garanich, J.S., Pahakis, M., Tarbell, J.M., 2005. Shear stress inhibits smooth muscle cell migration via nitric oxide-mediated

downregulation of matrix metalloproteinase-2 activity. Am. J. Physiol. Heart Circ. Physiol. 288 (5), H2244–H2252.
Haga, J.H., Li, Y.S., Chien, S., 2007. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. J. Biomech. 40 (5), 947–960.
Hamilton, D.W., Maul, T.M., Vorp, D.A., 2004. Characterization of the response of bone marrow derived progenitor cells to cyclic strain: implications for vascular tissue engineering applications. Tissue 10 (3/4), 361–370.
Harting, M., Jimenez, F., Pati, S., Baumgartner, J., Cox Jr., C., 2008. Immunophenotype characterization of rat mesenchymal stromal cells. Cytotherapy 10 (3), 243–253.
Hong, H.S., Kim, Y.H., Son, Y., 2012. Perspectives on mesenchymal stem cells: tissue repair, immune modulation, and tumor homing. Arch. Pharm. Res. 35 (2), 201–211.
Howe, A.K., Aplin, A.E., Juliano, R.L., 2002. Anchorage-dependent ERK signaling—mechanisms and consequences. Curr. Opin. Genet. Dev. 12 (1), 30–35.
Hsia, D.A., Mitra, S.K., Hauck, C.R., Streblow, D.N., Nelson, J.A., Ilic, D., Huang, S., Li, E., Nemerow, G.R., Leng, J., Spencer, K.S., Cheresh, D.A., Schlaepfer, D.D., 2003. Differential regulation of cell motility and invasion by FAK. J. Cell Biol. 160 (5), 753–767.
Ilić, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., Yamamoto, T., 1995. Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. Nature 377 (6549), 539–544.
Kang, M.N., Yoon, H.H., Seo, Y.K., 2012. Effect of mechanical stimulation on the differentiation of cord stem cells. Connect. Tissue Res. 53 (2), 149–159.
Kearney, E.M., Farrell, E., Prendergast, P.J., Campbell, V.A., 2010. Tensile strain as a regulator of mesenchymal stem cell osteogenesis. Ann. Biomed. Eng. 38 (5), 1767–1779.
Kim, K.M., Choi, Y.J., Hwang, J.H., Kim, A.R., Cho, H.J., Hwang, E.S., Park, J.Y., Lee, S.H., Hong, J.H., 2014. Shear stress induced by an interstitial level of slow flow increases the osteogenic differentiation of mesenchymal stem cells through TAZ activation. PLoS One 9 (3), e92427.
Li, S., Kim, M., Hu, Y.L., Jalali, S., Schlaepfer, D.D., Hunter, T., Chien, S., Shyy, J.Y., 1997. Fluid shear stress activation of focal adhesion kinase. Linking to mitogen-activated protein kinases. J. Biol. Chem. 272 (48), 30455–30462.
Li, M., Li, Z., Sun, X., 2008. Statins suppress MMP2 secretion via inactivation of RhoA/ROCK pathway in pulmonary vascular smooth muscles cells. Eur. J. Pharmacol. 591 (1–3), 219–223.
Li, Z., Li, C., Du, L., Zhou, Y., Wu, W., 2013. Human chorionic gonadotropin β induces migration and invasion via activating ERK1/2 and MMP-2 in humanprostate cancer DU145 cells. PLoS One 8 (2), e54592.
Lim, S.T., Mikolon, D., Stupack, D.G., Schlaepfer, D.D., 2008. FERM control of FAK function: implications for cancer therapy. Cell Cycle 7 (15), 2306–2314.
Lin, S.H., Shih, Y.W., 2014. Antitumor effects of the flavone chalcone: inhibition of invasion and migration through the FAK/JNK signaling pathway in human gastric adenocarcinoma AGS cells. Mol. Cell. Biochem. 391 (1–2), 47–58.
Natarajan, M., Hecker, T.P., Gladson, C.L., 2003. FAK signaling in anaplastic astrocytoma and glioblastoma tumors. Cancer J. 9 (2), 126–133.
Newby, A.C., 2006. Matrix metalloproteinases regulate migration, proliferation, and death of vascular smooth muscle cells by degrading matrix and non-matrix substrates. Cardiovasc. Res. 69 (3), 614–624.
Ocarino, N.M., Boeloni, J.N., Goes, A.M., Silva, J.F., Marubayashi, U., Serakides, R., 2008. Osteogenic differentiation of mesenchymal stem cells from osteopenic rats subjected to physical activity with and without nitric oxide synthase inhibition. Nitric Oxide 19 (4), 320–325.
Palumbo, R., Gaetano, C., Melillo, G., Toschi, E., Remuzzi, A., Capogrossi, M.C., 2000. Shear stress downregulation of platelet-derived growth factor receptor-beta and matrix metalloprotease-2 is associated with inhibition of smooth muscle cell invasion and migration. Circulation 102 (2), 225–230.
Park, S.J., Jeon, Y.J., 2012. Dieckol from Ecklonia cava suppresses the migration and invasion of HT1080 cells by inhibiting the focal adhesion kinase pathway downstream of Rac1-ROS signaling. Mol. Cells 33 (2), 141–149.
Park, J.S., Chu, J.S., Cheng, C., Chen, F., Chen, D., Li, S., 2004. Differential effects of equiaxial and uniaxial strain on mesenchymal stem cells. Biotechnol. Bioeng. 88 (3), 359–368.
Reis, S.T., Pontes-Junior, J., Antunes, A.A., de Sousa-Canavez, J.M., Dall'Oglio, M.F., Passerotti, C.C., Abe, D.K., Crippa, A., da Cruz, J.A., Timoszczuk, L.M., Srougi, M., Leite, K.R., 2011. MMP-9 overexpression due to TIMP-1 and RECK underexpression is associated with prognosis in prostate cancer. Int. J. Biol. Markers 26 (4), 255–261.
Ridley, A.J., Schwartz, M.A., Burridge, K., Firtel, R.A., Ginsberg, M.H., Borisy, G., Parsons, J.T., Horwitz, A.R., 2003. Cell migration: integrating signals from front to back. Science 302 (5651), 1704–1709.
Sacchetti, B., Funari, A., Michienzi, S., Di Cesare, S., Piersanti, S., Saggio, I., Tagliafico, E., Ferrari, S., Robey, P.G., Riminucci, M., Bianco, P., 2007. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131 (2), 324–336.
Salem, H.K., Thiemermann, C., 2010. Mesenchymal stromal cells: current understanding and clinical status. Stem Cells 28 (3), 585–596.
Stricker, J., Falzone, T., Gardel, M.L., 2010. Mechanics of the F-actin cytoskeleton. J. Biomech. 43 (1), 9–14.
Tan, C.T., Chu, C.Y., Lu, Y.C., Chang, C.C., Lin, B.R., Wu, H.H., Liu, H.L., Cha, S.T., Prakash, E., Ko, J.Y., Kuo, M.L., 2008. CXCL12/CXCR4 promotes laryngeal and hypopharyngeal squamous cell carcinoma metastasis through MMP-13-dependent invasion via the ERK1/2/AP-1 pathway. Carcinogenesis 29 (8), 1519–1527.
Yamane, T., Mitsumata, M., Yamaguchi, N., Nakazawa, T., Mochizuki, K., Kondo, T., Kawasaki, T., Murata, S., Yoshida, Y., Katoh, R., 2010. Laminar high shear stress up-regulates type IV collagen synthesis and down-regulates MMP-2 secretion in endothelium. A quantitative analysis. Cell Tissue Res. 340 (3), 471–479.
Yang, N., Hui, L., Wang, Y., Yang, H., Jiang, X., 2014. SOX2 promotes the migration and invasion of laryngeal cancer cells by induction of MMP-2 via the PI3K/Akt/mTOR pathway. Oncol. Rep. 31 (6), 2651–2659.
Yuan, L., Luo, Q., Yang, L., Song, G., 2012a. Role of FAK-ERK1/2 signaling pathway in proliferation of rat bone-marrow mesenchymal stem cells stimulated by cyclic stretching. J. Med. Biol. Eng. 33 (2), 229–238.
Yuan, L., Sakamoto, N., Song, G., Sato, M., 2012b. Migration of human mesenchymal stem cells under low shear stress mediated by mitogen-activated protein kinase signaling. Stem Cells Dev. 21 (13), 2520–2530.
Zheng, L., Huang, Y., Song, W., Gong, X., Liu, M., Jia, X., Zhou, G., Chen, L., Li, A., Fan, Y., 2012. Fluid shear stress regulates metalloproteinase-1 and 2 in human periodontal ligament cells: involvement of extracellular signal-regulated kinase (ERK) and P38 signaling pathways. J. Biomech. 45 (14), 2368–2375.