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Effect of Focal Adhesion Kinase on the Regulation of Realignment and Tenogenic Differentiation of Human Mesenchymal Stem Cells by Mechanical Stretch

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Abstract

Focal adhesion kinase (FAK) is a focal adhesion-associated protein kinase involved in cell adhesion and spreading. It is recruited as a participant in focal adhesion dynamics between cells and has a role in cell motility, differentiation, and survival. The role of FAK in the differentiation of human mesenchymal stem cells (hMSCs), however, is not well understood, particularly in terms of tenogenic differentiation. In this study, we reported that FAK regulates the mechanical stretch-induced realignment of hMSCs. We showed that FAK can be activated by mechanical stretch and, with a 10 μM PF 573228 (a novel small molecule inhibitor of FAK) treatment, FAK autophosphorylation at Tyr397 is significantly decreased. Moreover, our findings demonstrated that this decrease in FAK autophosphorylation at Tyr397 leads to the attenuation of upregulation of mechanical stretch-induced mRNA expression of tendon-related genes, including type I collagen, type III collagen, tenascin-C, and scleraxis. These results indicate that the FAK signaling molecule plays an important role in regulating cell realignment and tenogenic differentiation of hMSCs when induced by mechanical stretch. Collectively, our findings provide novel insight into the role of FAK in the realignment and mechanotransduction of hMSCs during the process of tenogenic differentiation induced by mechanical stretch.

Keywords: focal adhesion kinase, human mesenchymal stem cells, tenogenic, differentiation, mechanical stretch, signaling molecule

INTRODUCTION

Bone marrow mesenchymal stem cells (MSCs) are a kind of cells that have proliferation and multi-differentiation abilities [1]. Under some special biochemical conditions, MSCs are able to differentiate into osteogenic [2], chrondogenic [3], adipogenic [4], cardiogenic [5], neurogenic [6], and tenogenic [7]. Moreover, some studies have been carried out on the differentiation of MSCs by mechanical stimuli and have shown that MSCs can be differentiated into osteoblasts [8], chondrocytes [9], and smooth muscle cells [10]. It was also reported that MSCs can be induced to differentiate into tenogenic by mechanical stimuli [11]. This plasticity of MSCs gives us a new chance for healing of tendon injury; however, the mechanotransduction mechanism which regulates the differentiation of human mesenchymal stem cells (hMSCs) in bone marrow is still not clearly understood, especially for the tenogenic differentiation induced by mechanical stimuli.

Supramolecular focal adhesion complexes (FAC), which are assembled in response to integrins binding to extracellular matrix (ECM), are essential to sense extracellular signals and integrate the signals into the cellular responses [12]. Among the FAC, focal adhesion kinase (FAK) is one of the most important star molecules. FAK, a non-receptor protein tyrosine kinase, is constitutively associated with β-integrin of integrin receptors in virtually all cell types. The binding to integrins of components of the ECMs leads to the activation of FAK. FAK activation is demonstrated by an increase of autophosphorylation at Tyr397 as well as other sites of the protein. When FAK is activated, it autophosphorylates and binds to Src kinase which in turn phosphorylates other sites of FAK and the FAK-binding proteins, Cas and paxillin. Phosphorylated FAK becomes a docking site within FAC for mediators of multiple signaling events that regulate growth, survival, and morphogenesis [13].

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Furthermore, FAK is a critical signaling molecule for differentiation in many cell lineages. It was reported that FAK inhibition blocks osteogenic differentiation of hMSCs and FAK is an important mediator of laminin-5-induced osteogenic differentiation of hMSCs [14]. It was also demonstrated that FAK participates in adipocyte differentiation [15], and activation of the FAK/Extracellular signal-regulated kinase (ERK) signaling cascade by β1-integrin is involved in the differentiation and survival of human fetal pancreatic islet cells [16]. FAK is addressed to have a possible role in neuronal differentiation [17]. In addition, FAK is involved in the regulation of cell growth and survival through activation of PI3K/PKD1/Akt/PKB and Grb2/SOS/Ras/Raf-1/MEK/ERK pathways and it mediates cells motility and adhesion turnover through regulation of the Rho GTPases, especially RhoA, Rac-1, and Cdc42 [18]. FAK downregulates stress fiber formation mediated by RhoA and upregulates the formation of lamellipodia by activating Rac-1 via a Cas-Crk-DOCK-ELMO complex [12].

PF 573228 (hereafter referred as PF 228), a novel small molecule inhibitor, can inhibit FAK catalytic activity. The inhibitor interacts with FAK in the ATP-binding pocket and effectively blocks the catalytic activity of recombinant FAK protein or endogenous FAK expressed in a variety of normal and cancer cell lineages [19]. Treatment of cultured cells with the inhibitor significantly inhibits FAK autophosphorylation at Tyr397 and fails to inhibit growth or apoptosis. Additionally, similar treatment of cells with PF 228 results in inhibition of serum or fibronectin-directed migration and decreases focal adhesion turnover [19]. Matthew [20] and his colleagues have demonstrated that defects in platelet spreading and aggregation, intracellular calcium mobilization, and dense granule secretion when platelets are pre-treated with PF 228 are, in part, consistent with data obtained from the hematopoietic cell type-specific FAK knockouts. It implies that PF 228 can be used as a powerful tool for the research of the role of FAK in cultures.

Type I collagen (Col I), type III collagen (Col III), tenascin-C (TNC), and scleraxis (SCX) are tendon-related genes. Col I and Col III are often chosen as the primary reference proteins because they are the predominant constituents of native tendon tissue. TNC, an extracellular protein, is an excellent marker for tendon development, has been used extensively to detect early tendon primordia, and is also found within differentiated tendons. SCX, a new unique early marker for tendon fibroblasts, is a basic helix–loop–helix transcription factor expressed specifically and exclusively in tendons [21]. Therefore, expression of the four tendon-related genes was commonly used to characterize tenogenic differentiation [22,23]. Our previous work also manifested that mechanical stretch can induce tenogenic differentiation of MSCs [24]. However, the mechanism of mechanical stretch-induced tenogenic differentiation of hMSCs is still unknown. We supposed that FAK regulates the mechanical stretch-induced tenogenic differentiation of hMSCs. In this study, we examined the influence of mechanical stretch and PF 228 on the alignment of hMSCs. Additionally, we examined the influence of mechanical stretch and PF 228 on the autophosphorylation of FAK at Tyr397. Finally, we determined if FAK regulates mechanical stretch-induced tenogenic differentiation of hMSCs. Our findings suggest that FAK regulates mechanical stretch-induced realignment of hMSCs. Moreover, PF 228 attenuates mechanical stretch-induced increase of the autophosphorylation of FAK on Tyr397. Finally, PF 228 inhibits mechanical stretch-induced tenogenic differentiation of hMSCs.

MATERIALS AND METHODS

Cell Culture

hMSCs (UE6E7T-3) were acquired from Health Science Research Resources Bank, Osaka, Japan, and cultured in MSC growth medium (Invitrogen, Carlsbad, CA, USA) in 25 cm^2 culture flasks (Becton Dickinson Labware, Billerica, MA, USA) at an initial density of 1×10^4 cells/cm^2 for expansion without differentiation. Cells were kept in humidified incubator (SANYO, Osaka, Japan) at 37°C and supplemented with 5% CO_2. The culture medium was changed every 3 days. After reaching confluence (usually about 5 or 7 days) cells were released with 0.25% trypsin/1 mM EDTA (B3232, Takara Bio Inc., Otsu, Japan) and subcultured in 25 cm^2 culture flasks.

Cyclic Mechanical Stretch

Mechanical cell strain instrument (Model ST-140, STREX Co., Ltd, Osaka, Japan) consist of a control unit, a strain unit, and rectangular, and elastic silicone chambers. During stretch experiments, only strain unit was put into incubator. The chambers were used in the strain unit driven by an eccentric motor that allowed variation in magnitude (2–20%) and frequency (0.01–1.5 Hz) of the applied strain [25].

hMSCs were plated into chambers at an initial density of 1×10^4 cells/cm^2 (10 cm^2, STREX Co.), pre-coated with 1 μg/cm^2 human fibronectin (R & D, Minneapolis, MN, USA), and allowed to culture in incubator for 24 hr. The chambers were mounted on strain unit of the strain apparatus by four holes for hook, one end of the chambers was firmly attached to a fixed frame, whereas the other end was held on a movable frame. The movable frame was connected to a motor-driven shaft. The cells in the silicon chambers were exposed to a stretch treatment at amplitude of 10% and frequency of 1 Hz for 48 hr (the following mechanical stretch in this study is under the same condition if not mentioned). As control, static cells were grown on pre-coated chambers, but did not experience any stretch. For blockage of FAK autophosphorylation, hMSCs were incubated with PF 228 (Tocris,

Ellisville, MO, USA), dissolved in DMSO, for 1 hr before applying mechanical stretch.

Measurement of Cell Shape Index

Immediately after mechanical stretch, we photographed cells on each membrane randomly. The cell perimeter and area were measured with Fluoview 5.0 (Olympus, Tokyo, Japan). The shape indexes (SI) of hMSCs were calculated from the following formula:

$$\mathrm{SI} = \frac{4\pi A}{P^2}$$

where A and P are the area and perimeter, respectively, of a cell. The SI is a measurement of how circular or linear an object is. The SI is ranged from 0 to 1, and the larger the value of the SI, the more circular the object is.

Total RNA isolation and reverse transcription

Cells were lysed with 350 μl of lysate of Rneasy Mini Kit (Qiagen, Duesseldorf, Germany) per chamber. Total RNA was extracted according to the protocol of the kit. The RNA was resuspended in 50 μl of nuclease-free H_2O and analyzed by UV spectrophotometer to confirm the purity ($A_{260}/A_{280} \geq 1.80$). Reverse transcription was performed using High Capacity RNA-to-cDNA Kit (ABI, Carlsbad, CA, USA) with about 1.5 μg total RNA in a final 20 μl reaction volume.

Real-time reverse transcription quantitative polymerase chain reaction

Real-time polymerase chain reactions were performed with Taqman Gene Expression Master Mix (ABI) on ABI 7300 Real-Time PCR system. Predesigned Minor groove binder (MGB) probes of glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Hs99999905_ml), type I collagen (Col I, Hs01028956_ml), type III collagen (Col III, Hs00164103_ml), tenascin-C (TNC, Hs00233648_ml), and scleraxis (SCX, Hs03054634_gl) (ABI) were used to detect relative mRNA expressions. The expression levels of Col I, Col III, TNC, and SCX were analyzed with GAPDH as an internal control and were normalized to GADPH and calculated using standard curve method.

Western blot

Cell lysates were collected at the termination of each experiment from each culture condition. Briefly, cells were washed with PBS, then 100 μl of detergent-based lysis buffer (M-PER Mammalian Protein Extraction Reagent, Pierce, Rockford, IL, USA), protease inhibitor PMSF, and cocktail of phosphatase inhibitors (Pierce, 1:100 dilution) were added to each chamber for collection of total cellular proteins. Equal amounts of proteins (5–15 μg) from each sample were loaded into 8% SDS-PAGE gel for gel electrophoresis. The separated proteins were transferred to a PVDF membrane (Biorad, Hercules, CA, USA). The membrane was blocked in 5% BSA/TBS-Tween 20 solution at 4°C overnight followed by the application of monoclonal antibody specific for p-FAK (pY397) (rabbit IgG, Epitomics, Burlingame, CA, USA) or FAK (rabbit IgG, Epitomics) at 1:1000, in 5% BSA/TBS-Tween 20. After overnight with primary antibody at 4°C, the secondary antibody, anti-rabbit IgG-HRP (Cell Signaling Technology, Beverly, MA, USA) at 1:10,000 in 5% BSA/TBS-Tween 20, was applied for 1 hr at room temperature. The membrane was washed three times with 0.1% TBS/Tween 20 for 10 min after each antibody application. The proteins on the PVDF membrane were detected with the ECL detection system (Pierce), according to the manufacturer's protocol. The protein bands were quantified by volume summation of image pixels with Fujifilm LAS-4000 (Fujifilm, Tokyo, Japan).

Statistical Analysis

Means and standard deviations were reported for three single repeat samples. Paired Student t-test was performed, and a p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Influence of Mechanical Stretch and PF 228 on hMSCs Realignment

To determine the effect of FAK on the realignment of hMSCs driven by mechanical stretch, four experiment groups were designed. Control group (Con) did not experience mechanical stretch; PF 228 blocking group (PF) did not experience mechanical stretch, but was treated with 10 μM PF 228; stretch group (Str) experienced mechanical stretch only; stretch plus 10 μM PF 228 treatment group (Str+PF) not only experienced mechanical stretch, but also was treated with 10 μM PF 228. The DMSO concentration of each group was 0.1% (v/v). The mechanical stretch was performed as described above.

At the termination of mechanical stretch, the morphology of each group was observed by optical microscope (Figure 1). hMSCs of Con in monolayer appeared like typical fibroblast-like cells. They exhibited long cytoplasmic processes and spindle-shaped nucleus. Orientation of Con was random (Figure 1A). Orientation of PF was also random, and no significant orientation change was observed when compared with Con (Figure 1B). However, orientation of Str was different from Con or PF. hMSCs of Str showed perpendicular alignment with the direction of stretch (Figure 1C). Interestingly, as shown in Figure 1D, when hMSCs were treated with 10 μM PF 228 and exposed to mechanical stretch, the cell shape was significantly changed while the orientation was still random. It was observed that hMSCs were changed to stellate-like cells.

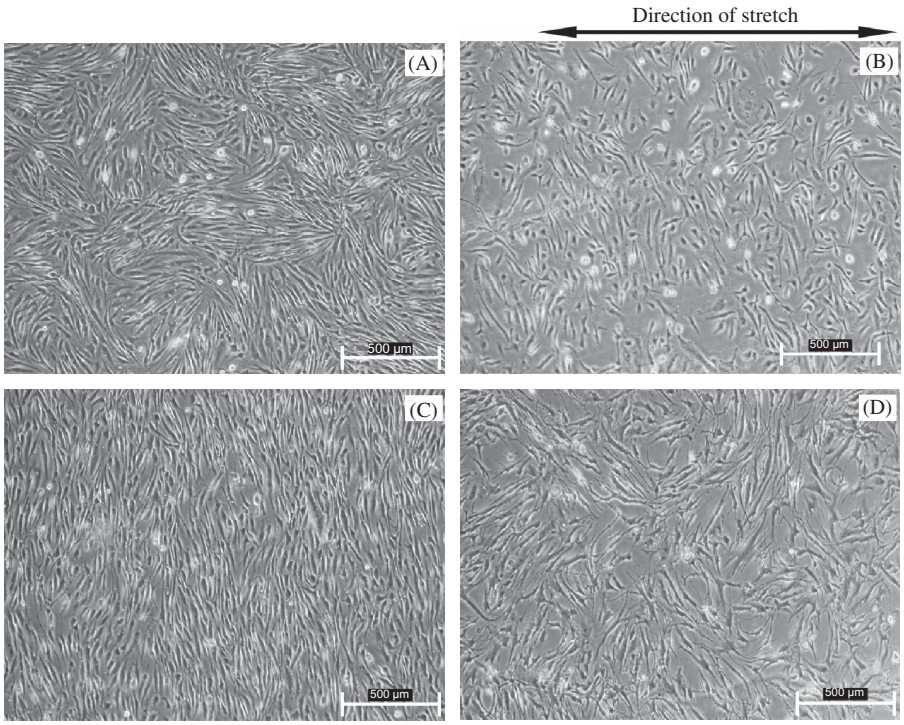

Figure 1. The cell morphology of the each group after 48 hr without or with treatment of stretch and/or PF 228. (A) hMSCs of control group (Con) presented random orientation; (B) hMSCs of PF 228 blocking group (PF) exhibited random orientation; (C) hMSCs of stretch group (Str) showed perpendicular alignment with respect to the direction of stretch; (D) hMSCs of stretch plus 10 μM PF 228 treatment group (Str+PF) had random orientation and the cell edge became rough, moreover, they became stellate-like cells.

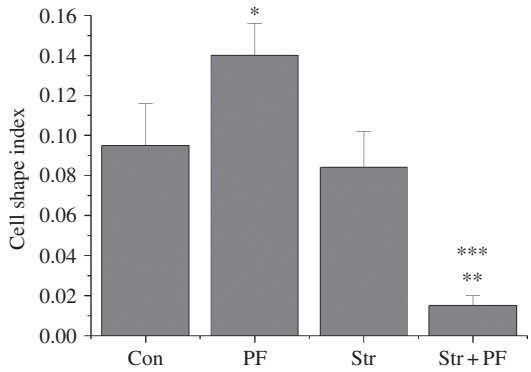

Figure 2. The cell shape index in each group. For cell shape index, 20 cells were chosen randomly and analyzed. "Con" indicates hMSCs of control group. "PF" indicates hMSCs of PF 228 blocking group. "Str" indicates hMSCs of stretch group. "Str+PF" indicates hMSCs of stretch plus 10 μM PF 228 treatment group. Data represent means ± standard deviations of three separate experiments (n = 3). "*" and "**" represent $p < 0.05$ and $p < 0.01$ versus Con, respectively; "***" means $p < 0.01$ versus PF.

Influence of Mechanical Stretch and PF 228 on Cell Shape Index

To quantitatively describe the cell shape shown in Figure 1, cell SI was introduced to describe how circular or linear hMSCs are. As shown in Figure 2, the cell SI of Str became smaller than that of Con, although there was no significant difference from the statistical perspective compared with Con. It indicates hMSCs of Str became slenderer. For hMSCs of PF, the cell SI became larger than before. It means hMSCs became more circular by treatment with 10 μM PF 228. Interestingly, the cell SI of Str+PF became much smaller than that of Con, Str, or PF. This is because the cell shape of Str+PF became much more irregular and the cells were changed to stellate-like cells from fibroblast-like.

Activation of FAK Affected by Mechanical Stretch and PF 228

To determine the effect of mechanical stretch and PF 228 on activation of FAK, hMSCs were prepared as described above, and then cultured with serum-free medium for 24 hr to synchronize cell cycle. Before applying the mechanical stretch, treatment of cell with increasing concentrations (0 , 0.1, 1, 3, 10 μM) of PF 228 for 1 hr to block FAK autophosphorylation at Tyr397 was performed. Hereafter, cells were stretched for a period of 30 min (1 Hz and 10% strain). hMSCs of control were maintained identically except for the application of stretch and PF 228. At the termination of experiment, all the hMSCs were lysed and the FAK autophosphorylation at Tyr397 was investigated by western blot as described above.

With experience of stretch for 30 min, the FAK autophosphorylation at Tyr397 was increased up to 1.766 ± 0.365 times compared with hMSCs of control

(Figure 3). It indicates that mechanical stretch can significantly promote the autophosphorylation of FAK at Tyr397 in hMSCs. The blocking effect of PF 228 on the autophosphorylation of FAK at Tyr397 was measured at the same time. Treatment of hMSCs with different concentration of PF 228 can significantly inhibit the FAK autophosphorylation at Tyr397. The results revealed that approximately 30% inhibition was routinely achieved with 3 μM PF 228 and approximately 75% inhibition with 10 μM (Figure 3) by comparing with stretched hMSCs without PF 228 treatment.

Influence of mRNA Expression by Mechanical Stretch and PF 228

To investigate the role of FAK signaling molecule in the process of tenogenic differentiation of hMSCs induced by mechanical stretch, 10 μM PF 228 was used to inhibit the FAK autophosphorylation at Tyr397. Three experiment groups were designed as follows: control group (Con) experienced mechanical stretch; stretch group (Str) experienced mechanical stretch only; stretch plus 10 μM PF 228 treatment group (Str+PF) not only experienced mechanical stretch, but also was treated with 10 μM PF 228. At the termination of the experiment, relative mRNA expressions of these cultures were analyzed by real-time RT-PCR as described above.

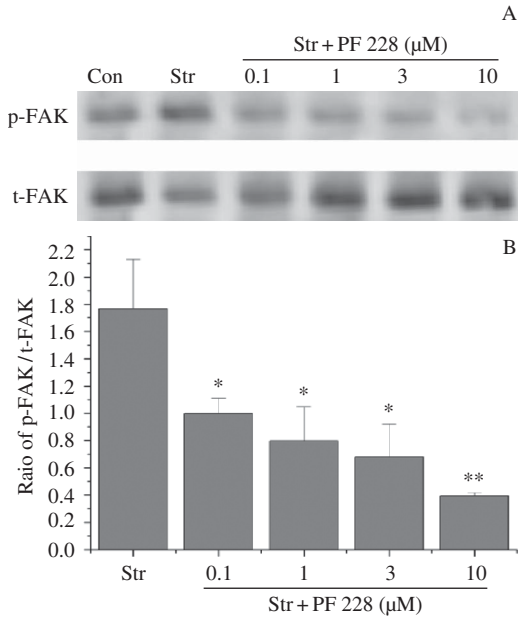

Figure 3. Inhibition of FAK autophosphorylation at Tyr397 by PF 228. (A) hMSCs were treated with the indicated concentrations of PF 228 (using serially diluted concentrations of inhibitor) incubated for 1 hr and then stretched for 30 min. Subsequently, the whole cell lysates were separated by SDS-PAGE and blotted with anti-phospho-FAK Tyr397 (upper panel) and anti-FAK (lower panel); (B) phosphorylation levels of FAK Tyr397 were normalized to total FAK of each sample. The phosphorylation levels of FAK Tyr397 of control group were normalized to 1. "Con" indicates hMSCs without treatment of stretch or PF 228; "Str" indicates hMSCs with stretch. Data represent means ± standard deviations of three separate experiments (n = 3). "*" and "**" represent $p < 0.05$ and $p < 0.01$ versus stretch group, respectively.

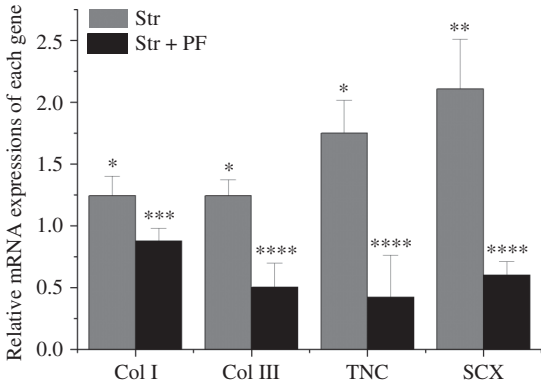

Figure 4. Relative mRNA expressions of each sample. For each sample, the mRNA expression levels of Col I, Col III, TNC, and SCX were normalized to GAPDH. The mRNA expression level of Col I, Col III, TNC, and SCX in control group were normalized to 1. Proportional fold change in transcript levels of stretch group (Str) and hMSCs of stretch plus 10 μM PF 228 treatment group (Str+PF) was expressed relative to static control. Data represent means ± standard deviations of three separate experiments (n = 3). Mean of relative mRNA expression less than 1 indicates that the gene expression level is decreased compared with that of control group; mean of relative mRNA expression more than 1 indicates that the gene expression level is increased compared with that of control group. "*" and "**" represent $p < 0.05$ and $p < 0.01$, respectively, mRNA expressions of Str versus respective Con. "***" and "****" represent $p < 0.05$ and $p < 0.01$, respectively, mRNA expressions of Str+PF versus respective Str.

mRNA expression levels were quantified for Col I, Col III, TNC, and SCX which were normalized to internal control gene, GAPDH. The mRNA expression levels of these genes of Con were normalized to 1. As shown in Figure 4, mechanical stretch and PF 228 have significant influence on mRNA expressions of Col I, Col III, TNC, and SCX. Compared with Con, mRNA expressions of Col I (up to 1.243 ± 0.159 times), Col III (up to 1.242 ± 0.129 times), and TNC (up to 1.749 ± 0.266 times) were upregulated significantly after stretch for 48 hr. SCX, a helix–loop–helix transcription factor, was increased to 2.107 ± 0.401 times by mechanical stretch. Interestingly, the results showed that the mechanical stretch-induced upregulation of the mRNA expressions of the four genes was attenuated by blocking FAK autophosphorylation at Tyr397 with 10 μM PF 228. Specifically, Col I, Col III, TNC, and SCX decreased to 0.876 ± 0.105 times, 0.504 ± 0.194 times, 0.42 ± 0.341 times, and 0.600 ± 0.110 times, respectively.

DISCUSSION

Mechanical stretch and PF 228 have important influence on realignment and cell SI. Mechanical stretch induces hMSCs to align perpendicular to the direction

of mechanical stretch and become slenderer than before. When hMSCs were exposed to mechanical stretch, the stress fibril experienced extracellular force. To resist the force, the stress fibril tends to align perpendicular to the direction of stretch and then resulted in realignment of hMSCs. At the same time, hMSCs became more circular by treatment with PF 228 only, whereas PF 228-treated hMSCs which were exposed to mechanical stretch did not realign and were changed to stellate-like cells from fibroblast-like. However, there is no evidence to illustrate why PF 228-treated hMSCs become stellate-like cells when exposed to mechanical stretch. The reason may be that mechanical stretch drives hMSCs to align perpendicular to the direction of stretch, whereas PF 228 blocks the realignment by inhibiting FAK autophosphorylation at Tyr397, which is essential to FAC turnover, cell spreading, and migration [26,27].

Our results demonstrate that mechanical stretch can induce rapid increase of the FAK autophosphorylation at Tyr397. It is consistent with a recent study which demonstrated that cyclic stretch activates FAK and RhoA via β_{1D}-integrin isoform in skeletal myoblasts [28]. It was also reported that RhoA/ROCK signaling pathway plays a crucial role in stretch-induced autophosphorylation of FAK at Tyr397, presumably by coordinating upstream events operationally linked to the actin cytoskeleton [29]. Furthermore, our results show that PF 228 can significantly block the FAK autophosphorylation at Tyr397 and the concentration of 10 μM PF 228 is appropriate to be used as a tool to analyze the function of FAK.

We also demonstrate that mechanical stretch can induce tenogenic differentiation of hMSCs; furthermore, the decrease of FAK autophosphorylation at Tyr397 blocked by 10 μM PF 228 leads to the attenuation of upregulated tenogenic gene expressions induced by mechanical stretch. It suggests that FAK senses the extracellular force and regulates the mechanical stretch-induced tenogenic differentiation of hMSCs. Our results show that mechanical stretch not only results in the changes in cell shape, but also induces the changes in gene expressions. McBeath et al. found that controlling cell shape regulates gene expressions and differentiation of hMSCs [30], while induced pluripotent stem cells (iPS cells) were generated from human fibroblast through transfection of Oct-3/4, SOX2, c-Myc, and klf4 genes which implied that changes in gene expression alter cell shape [31]. These findings together with our results suggest that there is strong correlation between changes in cell shape and changes in gene expression.

In addition, FAK pathway also plays important role in the differentiation of MSCs into other cell lineages. It was reported that FAK activation is an essential early step in regulating ECM-induced osteogenic differentiation of hMSCs [32]. Ward Jr [33] and his colleagues found that FAK signaling controls osteogenic differentiation through ERK1/2 pathway and it is a critical decision maker in ECM/strain-enhanced osteogenic differentiation. Tamura [34] and his colleagues demonstrated that FAK activity is essential for BMP-Smad signaling to stimulate osteoblastic differentiation. These studies further indicate FAK is crucial to convert extracellular signaling into intracellular signaling and triggers a cascade of downstream signaling pathway. The data presented here establish a link among mechanical stretch, FAK, and tenogenic differentiation. Taken together, FAK integrates those signal events, such as mechanical stretch, integrins, Rho GTPase family, cytoskeleton, and so on, then converts the mechanical signal into biochemical signal, and triggers the tenogenic differentiation of hMSCs. To our knowledge, this is the first demonstration that mechanical stretch induces hMSC differentiation through FAK signaling molecule.

Consequently, mechanical stretch can induce rapid increase of autophosphorylation of FAK at Tyr397, and PF 228 can be used as a useful tool to assess the role of FAK signaling molecule in hMSCs. Furthermore, FAK signaling molecule regulates the realignment of hMSCs driven by mechanical stretch, and it senses mechanical stretch and converts mechanical signal into biochemical signal through a network of downstream pathways in the process of mechanical stretch-induced tenogenic differentiation of hMSCs.

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REFERENCES

[1] Friedenstein, A.J., Petrakova, K.V., Kurolesova, A.I., and Frolova, G.P. (1968). Heterotopic transplants of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* 6(2):230–247.

[2] Salasznyk, R.M., Williams, W.A., Boskey, A., Batorsky, A., and Plopper, G.E. (2004). Adhesion to vitronectin and collagen I promotes osteogenic differentiation of human mesenchymal stem cells. *J. Biomed. Biotechnol.* 1:24–34.

[3] Mackay, A.M., Beck, S.C., Murphy, J.M., Barry, F.P., Chichester, C.O., and Pittenger, M.F. (1998). Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng.* 4(4):415–428.

[4] Jaiswal, R.K., Jaiswal, N., Bruder, S.P., Mbalaviele, G., Marshak, D.R., and Pittenger, M.F. (2000). Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. *J. Biol. Chem.* 275(13):9645–9652.

[5] Toma, C., Pittenger, M.F., Cahill, K.S., Byrne, B.J., and Kessler, P.D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 105(1):93–98.

[6] Cohen, L.A., and Guan, J.L. (2005). Mechanisms of focal adhesion kinase regulation. *Curr. Cancer Drug Targets* 5(8):629–643.

[7] Hoffmann, A., Pelled, G., Turgeman, G., Eberle, P., Zilberman, Y., Shinar, H., Keinan-Adamsky, K., Winkel, A., Shahab, S., Navon, G., Gross, G., and Gazit, D. (2006). Neotendon formation induced by manipulation of the Smad8 signalling pathway in mesenchymal stem cells. *J. Clin. Invest*. 116(4):940–952.

[8] Jagodzinski, M., Drescher, M., Zeichen, J., Hankemeier, S., Krettek, C., Bosch, U., and Griensven, M. (2004). Effects of cyclic longitudinal mechanical strain and dexamethasone on osteogenic differentiation of human bone marrow stromal cells. *Eur. Cells Mater*. 7:35–41.

[9] Huang, C.Y.C., Hagar, K.L., Frost, L.E., Sun, Y., and Cheung, H.S. (2004). Effects of cyclic compressive loading on chondrogenesis of rabbit bone-marrow derived mesenchymal stem cells. *Stem Cells* 22(3):313–323.

[10] Kurpinski, K., Chu, J., Hashi, C., and Li, S. (2006). Anisotropic mechanosensing by mesenchymal stem cells. *Proc. Natl Acad. Sci. USA* 103(44):16095–16100.

[11] Altman, G., Horan, R., Martin, I., Farhadi, J., Stark, P.R., Volloch, V., Richmond, J.C., Vunjak-Novakovic, G., and Kaplan, D.L. (2002). Cell differentiation by mechanical stress. *FASEB J.* 16:270–272.

[12] Parsons, J.T. (2003). Focal adhesion kinase: The first ten years. *J. Cell Sci*. 116(8):1409–1416.

[13] Ilic, D., Damsky, C.H., and Yamamoto, T. (1997). Focal adhesion kinase: At the crossroads of signal transduction. *J. Cell Sci*. 110(4):401–407.

[14] Salasznyk, R.M., Klees, R.F., Boskey, A., and Plopper, G.E. (2007). Activation of FAK is necessary for the osteogenic differentiation of human mesenchymal stem cells on laminin-5. *J. Cell. Biochem*. 100(2):499–514.

[15] Li, J.J., and Xie, D. (2007). Cleavage of focal adhesion kinase (FAK) is essential in adipocyte differentiation. *Biochem. Biophys. Res. Commun*. 357(3):648–654.

[16] Saleem, S., Li, J.M., Yee, S.P., Fellows, G.F., Goodyer, C.G., and Wang, R. (2009). Beta 1 integrin/FAK/ERK signalling pathway is essential for human fetal islet cell differentiation and survival. *J. Pathol*. 219(2):182–192.

[17] Park, S.Y., Avraham, H., and Avraham, S. (2000). Characterization of the tyrosine kinases RAFTK/Pyk2 and FAK in nerve growth factor-induced neuronal differentiation. *J. Biol. Chem*. 275(26):19768–19777.

[18] Mitra, S.K., Hanso, D.A., and Schlaepfer, D.D. (2005). Focal adhesion kinase: In command and control of cell motility. *Nat. Rev. Mol. Cell Biol*. 6(1):56–68.

[19] Slack-Davis, J.K., Martin, K.H., Tilghman, R.W., Iwanicki, M., Ung, E.J., Autry, C., Luzzio, M.J., Cooper, B., Kath, J.C., Roberts, W.G., and Parsons, J.T. (2007). Cellular characterization of a novel focal adhesion kinase inhibitor. *J. Biol. Chem*. 282(20):14845–14852.

[20] Jones, M.L., Shawe-Taylor, A.J., Williams, C.M., and Poole, A.W. (2009). Characterization of a novel focal adhesion kinase inhibitor in human platelets. *Biochem. Biophys. Res. Commun*. 389(1): 198–203.

[21] Schweitzer, R., Chyung, J.H., Murtaugh, L.C., Brent, A.E., Rosen, V., Olson, E.N., Lassar, A., and Tabin, C.J. (2001). Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. *Development* 128(19): 3855–3866.

[22] Kuo, C.K. and Tuan, R.S. (2008). Mechanoactive tenogenic differentiation of human mesenchymal stem cells. *Tissue Eng*. 14(10):1615–1627.

[23] Chen, Y.J., Huang, C.H., Lee, I.C., Lee, Y.T., Chen, M.H., and Young, T.H. (2008). Effects of cyclic mechanical stretching on the mRNA expression of tendon/ligament-related and osteoblast-specific genes in human mesenchymal stem cells. *Connect. Tissue Res*. 49(1):7–14.

[24] Song, G., Luo, Q., Xu, B., and Ju, Y. (2010). Mechanical stretch-induced changes in cell morphology and mRNA expression of tendon/ligament-associated genes in rat bone-marrow mesenchymal stem cells. *Mol. Cell. Biomech*. 7:165–174.

[25] Song, G.B., Ju, Y., Soyama, H., Ohashi, T., and Sato, M. (2007). Regulation of cyclic longitudinal mechanical stretch on proliferation of human bone marrow mesenchymal stem cells. *Mol. Cell. Biomech*. 4(4):201–210.

[26] Sieg, D.J., Hauck, C.R., Ilic, D., Klingbeil, C.K., Schaefer, E., Damsky, C.H., and Schlaepfer, D.D. (2000). FAK integrates growth-factor and integrin signals to promote cell migration. *Nat. Cell Biol*. 2(5):249–256.

[27] Gao, H., Priebe, W., Glod, J., and Banerjee, D. (2009). Activation of signal transducers and activators of transcription 3 and focal adhesion kinase by stromal cell-derived factor 1 is required for migration of human mesenchymal stem cells in response to tumor cell-conditioned medium. *Stem Cells* 27(4):857–865.

[28] Zhang, S.J., Truskey, G.A., and Kraus, W.E. (2007). Effect of cyclic stretch on β_{1D}-integrin expression and activation of FAK and RhoA. *Am. J. Physiol. Cell Physiol*. 292(6): C2057–C2069.

[29] Torsoni, A.S., Marin, T.M., Velloso, L.A., and Franchini, K.G. (2005). RhoA/ROCK signaling is critical to FAK activation by cyclic stretch in cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol*. 289(4):H1488–H1496.

[30] McBeath, R., Pirone, D.M., Nelson, C.M., Bhadriraju, K., and Chen, C.S. (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6(4):483–495.

[31] Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4):663–676.

[32] Salasznyk, R.M., Klees, R.F., Williams, W.A., Boskey, A., and Plopper, G.E. (2007). Focal adhesion kinase signaling pathways regulate the osteogenic differentiation of human mesenchymal stem cells. *Exp. Cell Res*. 313(1):23–27.

[33] Ward, D.F. Jr, Williams, W.A., Schapiro, N.E., Weber, G.L., Christy, S.R., Salt, M., Klees, R.F., Boskey, A., and Plopper, G.E. (2007). Focal adhesion kinase signaling controls cyclic tensile strain enhanced collagen I-induced osteogenic differentiation of human mesenchymal stem cells. *Mol. Cell. Biomech*. 4(4):177–188.

[34] Tamura, Y., Takeuchi, Y., Suzawa, M., Fukumoto, S., Kato, M., Miyazono, K., and Fujita, T. (2001). Focal adhesion kinase activity is required for bone morphogenetic protein-Smad1 signaling and osteoblastic differentiation in murine MC3T3-E1 cells. *J. Bone Miner. Res*. 16(10):1772–1779.