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Mechanical stretch inhibits adipogenesis and stimulates osteogenesis of adipose stem cells

X. Yang*,†, X. Cai*,†, J. Wang*, H. Tang*,†, Q. Yuan*, P. Gong*,† and Y. Lin*

**State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu, China and †Oral Implant Center, West China College of Stomatology, Sichuan University, Chengdu, China*

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Abstract

A reciprocal relationships between osteogenesis and adipogenesis has been observed *in vitro* and *in vivo*, and mechanical stretch has been believed to be a regulating factor of osteo-adipogenic axis differentiation of mesenchymal stem cells. In this study, rat adipose stem cells (ASCs) were isolated and cultured in adipogenic or normal medium. Their exposure to cyclic mechanical stretch (2000 με, 1 Hz) in the presence of adipogenic medium decreased mRNA and protein level of PPAR-γ, and increased Runx2 mRNA and protein levels as well as Pref-1 mRNA level, compared to static samples. ASCs cultured in normal medium without adipogenic induction did not show any significant change in mRNA expression of PPAR-γ, Runx2, nor Pref-1 irrespective of mechanical loading. Stretching induced phosphorylation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) during the induction period. It was concluded that mechanical stretch inhibited adipogenesis and stimulated osteogenesis of these ASCs in the presence of adipogenic medium and that ERK1/2 activation may be involved in the mechanical stress-induced trans-differentiation.

Introduction

The phenomenon of reciprocal decrease in osteogenesis and increase in adipogenesis in bone marrow cells, associated with aging (1,2) has been observed in clinical practice. *In vitro* studies also have shown that this reciprocal relationship exists between osteogenic and adipogenic differentiation (3). During the differentiation process, peroxisome proliferator-activated receptor-γ (PPAR-γ) and runt-related transcription factor 2 (Runx2) act as a core regulatory factors, modulating adipogenic and osteogenic differentiation, respectively. Our previous studies have revealed an inverse relationship between PPAR-γ and Runx2 expression in osteogenic and adipogenic differentiation, and decrease in osteogenesis correlated with increase in adipogenesis (4). These results suggested that adipocytes are generated at the expense of osteoblasts and that adipocytes and osteoblasts may originate from the same progenitors *in vivo*.

Mechanical force is a fundamental biological factor that stimulates fracture healing and bone remodelling processes (5), and most studies indicate that mechanical stress is an anabolic factor for osteogenic differentiation of bone mesenchymal stem cells (BMSCs) or osteoblasts (6,7). Results from our previous studies, along with those of other teams, have shown that cyclic tensile mechanical loading over a lengthy duration might promote expression of BMP-2 and Runx2, and thus osteogenic differentiation of adipose-derived stem cells (ASCs) in osteogenic medium (8). Moreover, we hypothesize that mechanical force is one of the pivots that modulate osteogenic differentiation, depending on the induction environment of progenitor cells. Although several studies have reported the trans-differentiation effect of mechanical stress on preadipocyte cell lines 3T3L-1 (9), BMSCs (10) and human umbilical cord perivascular cells (HUCPVC) (11), the response of ASCs to mechanical stretch under different induction conditions and related signalling pathways are still unclear.

Activation of extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) has also been demonstrated to be an important signalling pathway in differentiation of the preadipocyte cell line 3T3-L1 into mature adipocytes

Correspondence: Y. Lin, State Key Laboratory of Oral Diseases, West China College of Stomatology, Sichuan University, Chengdu 610041, China. Fax: 86-28-85503487; E-mail: yunfenglin@scu.edu.cn and P. Gong, Oral Implant Center, West China College of Stomatology, Sichuan University, Chengdu 610041, China. Tel: 86-28-85503571; Fax: 86-28-85503579; E-mail: gp602002@163.com

(9). ERK phosphorylation of PPAR-γ results in reduction in PPAR-γ transcriptional activity. Furthermore, previous studies have indicated that mechanical stress regulated osteogenesis through the MAPK pathway (12,13). Thus, we believe that ERK1/2 is a key signalling pathway in mechano-regulation of osteogenesis and adipogenesis of preadipocytes.

Although some studies have indicated that mechanical signals biased differentiation of mesenchymal cells towards osteogenesis over adipogenesis, few have manifested whether mechanical stress could trans-differentiate mesenchymal stem cells that undergo adipogenesis. In the present study, we show that the ASCs favour osteogenic differentiation when loaded with mechanical stretch over lengthy duration, even under adipogenic induction; however, without induction, ASCs maintain their undifferentiated state even loaded with mechanical stress. We also demonstrate that mechanical stretch inhibited adipogenic differentiation and promoted osteogenic differentiation of ASCs, thus initially conclude that ERK1/2 pathway may be involved in the regulation mechanism.

Materials and methods

Cell culture

ASCs were isolated as described by Zuk *et al.* (14) with minor modifications. Eighteen 4-week-old Sprague–Dawley rats were prepared, using standard sterile techniques, to excise inguinal fat pads, following protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Sichuan University. Tissue obtained was washed twice in phosphate-buffered saline supplemented with streptomycin sulphate and penicillin, incubated in α-modified Eagle's medium (α-MEM) and finely minced into small pieces of 0.5 cm^3. This fine tissue was digested by 0.05% type 1 collagenase, with vigorous shaking, for 40 min at 37 °C. Floating cell populations were removed by centrifugation at 1200 rpm (250 ***g***) for 8 min and cells were pelleted. Single-cell suspension was obtained and re-suspended in culture medium of α-MEM supplemented with antibiotics (penicillin–streptomycin solution), sodium bicarbonate and 10% foetal bovine serum (Gibco, Paisley, UK) and finally cells were seeded in the plastic flasks for the final isolation step selected for plastic adherent populations. Cells were cultured at 37 °C in 90% humidified atmosphere and 5% CO_2. Third passage was used for the following test: phenotypic profile of ASCs was investigated by immunofluorescene staining for STRO-1, and immunocytochemical staining for CD34, CD44 and CD90; cells were characterized as adipose-derived stem cells, as noted in our previous investigations (8).

Induction of adipogenic differentiation

Rat ASCs were transferred into adipogenic medium (1 μM dexamethasone, 10 μM insulin, 200 μM indomethacin, and 0.5 mM isobutyl-methylxanthine, and 10% FBS), 1×10^5/ml density. After 72 h induction, the cells reached 70% confluence and differentiated ones were stained with oil red-O and western blotting for PPAR-γ was performed to assess adipogenic differentiation. Control cells were maintained in standard medium and noted to be the non-induced control (NIC).

Application of cyclic tensile stretch

As previous studies have shown, application of cyclic stretching only during the late phase of induction inhibited adipo-differentiation of preadipocytes (9), thus we also applied mechanical tensile stretch after 3 days adipogenic induction. Cells were seeded on a polyethylene plastic plate (size 7.8 × 3.8 cm) cut off from its BD Falcon™ 75 cm^2 cell culture flask (BD Falcon, Franklin lakes, NJ) at density of 2×10^5 cells per plate. All cells cultured on the plastic plate were uniformly exposed to exactly the same tensile force. When cells were totally attached to the plate 6 h later, culture medium was replaced by adipogenic medium. A four-point bending mechanical loading device (Fig. 1) (15) was then used. Cells were randomly divided into four groups. Loading groups were exposed to uniaxial cyclic tensile stretch for 2000 με, 1 Hz for 6 and 2 h, respectively, after ASCs were adipo-induced for 72 h. ASCs that were retained in adipogenic medium without being loaded were used as induced-control group. In the third group, ASCs were exposed to mechanical loading under the same conditions, but without prior induction, and correspondingly, the non-induced control (NIC) group was kept in static medium without any

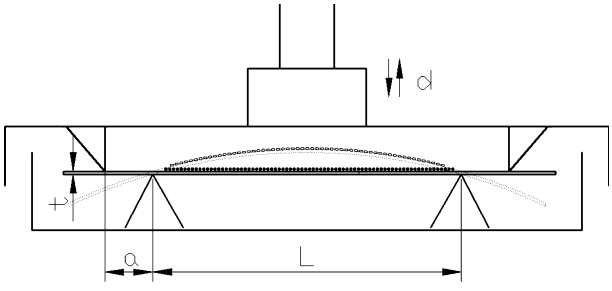

Figure 1. Illustrations of the four-point bending mechanical loading device. *L*, distance between two outer pressure points; *a*, distance between outer and inner pressure points; *t*, thickness of loading plate; *d*, distance of pressure point movement; strain(ε) of cells attached to upper side of loading plate was calculated according the following formula: $\varepsilon = td/a(L - 1.33a)$. In this experiment, ASCs were exposed to uniaxial cyclic tensile stretch for 2000 με, 1 Hz, which lasted 6 and 2 h.

mechanical loading. All measurements began 2 h after the last strain cycle.

Analysis of mRNA by real-time PCR

Total RNA of the rat ASCs was extracted using Total Tissue/Cell RNA Extraction Kits (Watson, China) according to the manufacturer's protocol. Total RNA integrity was verified by 1.2% agarose gel electrophoresis and yield and purity were confirmed by ratio of A (260)/A (280) using UV-spectroscopy. cDNA synthesis was performed using transcriptor reverse transcriptase (Takara Biotechnology, Shiga, Japan). To establish the standard curve of a certain gene, cDNA samples were amplified using an RT-PCR kit (Tiangen Biotech, Beijing, China); specifically designed primers are shown in Table 1. Real-time PCR was run in the ABI PRISM 7300 Sequence Detection system (Applied Biosystems, Foster City, CA, USA) using hotstart DNA Master SYBR Green I Kit (Takara Biotechnology, Co., Ltd) with the following program: 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by melting curve analysis. Specificities of PCR products were verified by melting curve analyses between 60 and 95 °C. For each reaction, a melting curve was generated to test primer dimer formation and false priming. Values of relative gene expression were normalized by house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Immunofluorescence staining

ASCs were seeded on loading plates, cultured with adipogenic medium and exposed to mechanical loading, or kept static as described above. Before staining, cells were washed briefly in PBS, fixed in 4% buffered paraformaldehyde for 30 min at room temperature and blocked in 0.5% bovine serum albumin (BSA) for 15 min. Cells were subsequently incubated overnight at 4 °C with rabbit anti-PPAR-γ polyclonal antibodies (1:100) (Abcam, Cambridge, UK) or rabbit anti-PPAR-γ monoclonal antibody with phosphoserine at residue 82 (1:100) (Upstate, Lake Placid, NY, USA), goat anti-Runx2 polyclonal antibodies (1:100) (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), rabbit anti-phosphor-ERK1/2 monoclonal antibodies (1:200) (Cell Signaling Technology, Beverly, MA, USA). Sequentially, specimens were incubated in secondary antibodies conjugated to Rhodamine (Pierce Biotechnology, Rockford, IL, USA) for 1 h at room temperature, and nuclei were stained with DAPI (Molecular Probes, Eugene, OH, USA) for 1 min. After rinsing in PBS, cells were observed and imaged using a DMi6000-B fluorescence microscope (Leica Microsystem, Wetzlar, Germany).

Western blot analysis

Before cell treatment, layers were washed three times in PBS buffer, and total proteins were extracted using Protein Extract Reagents (Pierce) supplemented with 1 mM PMSF, 1 mg/ml pepstatin and 1 mg/ml leupeptin, followed by centrifugation (4 °C, 13 000 ***g*** for 10 min) to remove cell debris. Protein concentration was assessed using BCA kit (Pierce) according to the manufacturer's instructions. Twenty micrograms of total protein were analysed by western blotting using anti-PPAR-γ (phosphor 112) antibody at 1:500 (Abcam), anti-Runx2 antibody at 1:500 (Santa), anti-phospho-ERK1/2 or anti-ERK1/2 antibody at 1:2000 (Cell signaling Technology) as per the manufacturer's instructions. Immunocomplexes were visualized using enhanced chemiluminescence reagent (Pierce) according to the manufacturer's instructions.

Statistical analysis

We performed three or more independent sets of experiments. Data were presented as means ± SD and analysed by the test of variance of paired data. $P < 0.05$ was considered statistically significant.

Results

Cyclic tensile stretch inhibited adipogenic differentiation of ASCs

The rat ASCs were sub-cultured on loading plates for 6 h, and were exposed to adipogenic medium. Adipogenic stem cells were induced for 72 h and cyclic tensile stretch with frequency of 1 Hz and magnitude of 2000 με was

Table 1. Primer sequences for real-time PCR assay

Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
PPAR-γ	TGCACTGCCTATGAGCACTT	TGATGTCAAAGGAATGCGAG
Runx-2	TTCGTCAGCGTCCTATCAGTTC	CTTCCATCAGCGTCAACACC
Pref-1	TGTCATGGAGTCTGCAAGG	CAAGCCCGAACGTCTATTTC
GAPDH	TATGACTCTACCCACGGCAAGT	ATACTCAGCACCAGCATCACC

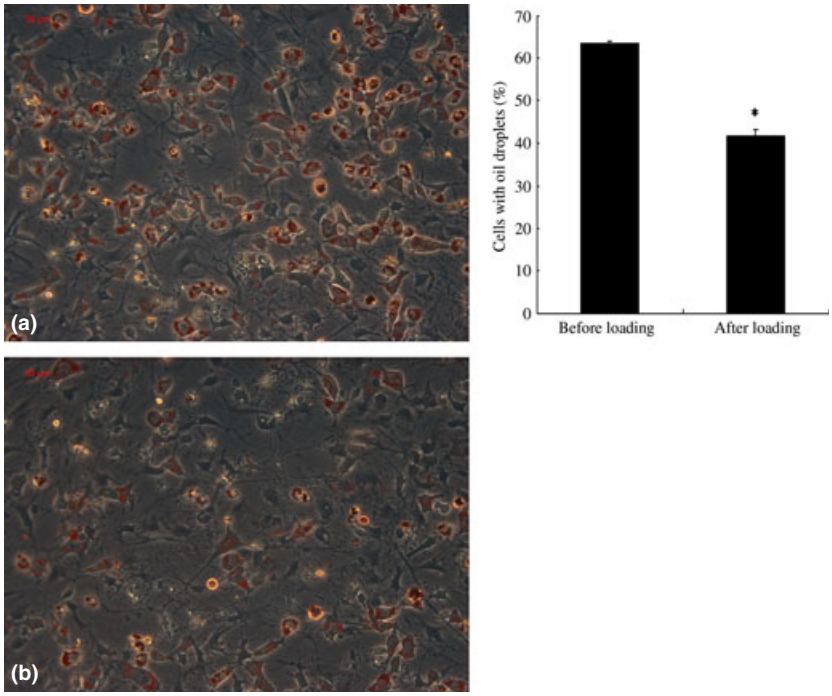

Figure 2. Mechanical stretch significantly reduced adipogenesis of ASCs. (a) Three days after adipogenic induction, lipid-filled cells, positive after oil red O staining, were observed under the light microscope. (b) Six hours mechanical stretching significantly reduced numbers of oil droplet-filled cells from 63% to 41%.

applied to them, undergoing adipogenic differentiation. Cytoplasmic lipid accumulation was visible after 72 h as demonstrated by positive oil red O staining in the induced-control group (Fig. 2a). However, ASCs cultured in adipogenic medium for 72 h while loaded with mechanical tension exhibited less lipid-laden cells compared to the induction control group (Fig. 2b).

Cyclic tensile stretch inhibited adipogenesis and stimulated osteogenesis

Pref-1 has been identified as a marker of undifferentiated preadipocytes, PPAR-γ is one of the most critical transactivators in adipogenesis initiation, while Runx-2 is recognized as a marker of transcriptional in initiation of osteogenesis. Thus, effects of mechanical tension loading on expression of these regulation and transcription factors was assessed by quantitative real-time polymerase chain reaction. ASCs were cultured with or without induction and mechanically loaded or kept static. Results showed that mRNA levels of PPAR-γ decreased significantly to 25% after loading for 6 h ($P < 0.01$) and 30% after loading for 2 h ($P < 0.01$) compared to static controls (Fig. 3a). In contrast, mRNA level of Runx2 increased 7-fold after 6-h mechanical loading ($P < 0.01$) and 4-fold after 2-h mechanical loading (Fig. 3b); Pref-1 significantly increased after 6-h loading ($P < 0.05$) (Fig. 3c). However, cells cultured in standard medium without induction showed no significant change in mRNA expression of PPAR-γ, Runx2, nor Pref-1, irrespective of mechanical loading (Fig. 3d–f).

As mechanical tension did not significantly change mRNA level of PPAR-γ, Runx2, or Pref-1 when cells were not exposed to adipogenic induction, we further examined protein levels of key factors in osteogenic and adipogenic differentiation when cells were kept in adipogenic medium, only. Protein expression levels of PPAR-γ and Runx2 were examined by both immunofluorescence staining and by western blotting. PPAR-γ protein expression increased significantly during adipogenic differentiation and cyclic stretch significantly reduced PPAR-γ at the protein level (Fig. 4). In contrast, Runx2 protein level was greatly increased after mechanical stretching (Fig. 5). Results indicated that mechanical tension induced down-regulation of PPAR protein level and upregulation of Runx2 protein, which was consistent with mRNA changes in PPAR-γ and Runx2 analysed by PCR (Fig. 3).

Cyclic tensile stretch activated the ERK/MAPK pathway

As previously demonstrated, ERK was the key factor that regulated the switch from osteogenesis to adipogenesis in human MSCs. Thus, we determined the function of ERK1/2 in mechanical tension-induced differentiation of

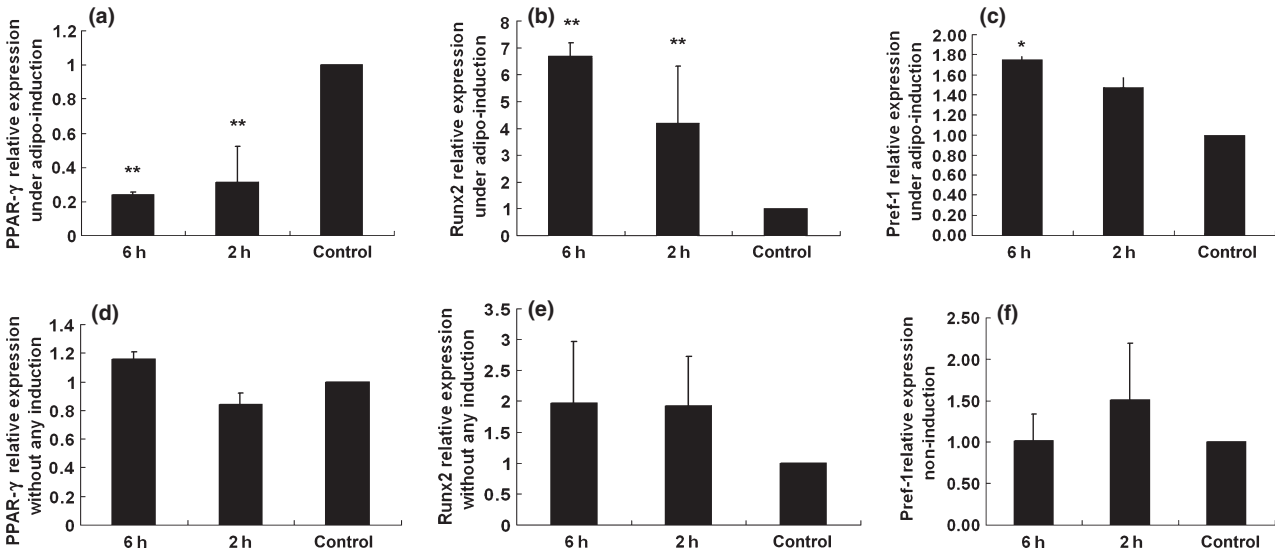

Figure 3. Mechanical stretch inhibited adipogenesis and promoted osteogenesis under adipogenic induction. After adipogenic induction for 72 h and treatment with uniaxial cyclic tensile stretch of 2000 με at 1 Hz, mRNA level of PPAR-γ reduced to 25% after loading for 6 h ($P < 0.01$) and 30% after loading for 2 h ($P < 0.01$) compared to static controls (a); Runx2 transcription increased 7-fold after 6-h mechanical loading ($P < 0.01$) and 4-fold after 2-h mechanical loading (b); Pref-1 significantly increased after 6-h loading ($P < 0.05$) (c); otherwise, when ASCs did not undergo adipogenic induction, PPAR-γ, Runx2 or Pref-1transcription did not significantly change irrespective of mechanical loading (d–f).

this cell type. We performed western blot analysis and immunofluorescence using anti-phosphorylated-ERK antibody, to examine effects of mechanical tension on phosphorylation of ERK1/2. Adipogenic induction activated ERK1/2 slightly (Fig. 6h); however, robust activation of ERK was observed when ASCs were loaded with mechanical tension compared to static controls, specially when this lasted for 6 h (Fig. 6h,i). Results of immunofluorescence staining confirmed increases in phosphorylation of ERK1/2 due to mechanical strain; moreover, activated ERK1/2 increased in cell nuclei of strained cultures compared to those of cells exposed to induction only, as shown by DAPI overlap in Fig. 6f.

Discussion

In clinical practice, it has been observed that there is an inverse relationship between bone marrow adiposity and amounts of bone in the axial skeleton (16). Given accumulating evidence for a reciprocal relationship between adipocytic and osteobalstic lineages, it appears that factors that drive differentiation towards one cell fate suppress the other (4,17). Our previous investigations have indicated that mechanical forces may promote osteoblastic differentiation of ASCs (8). This study has focused on the role of mechanical tensile stretch in reciprocal osteo- and adipodifferentiation of ASCs, and found that adipogenic-induced ASCs can be trans-differentiated, biasing adipocytic lineage and favouring osteogenic differentiation, and that ERK1/2 pathway may be involved in the regulation mechanism.

We have previously shown that cyclic mechanical stress can improve osteogenesis of ASCs under osteogenic-inducing conditions in a duration-dependent manner, and 6 consecutive hours stress can significantly upregulate mRNA expression of osteogenic molecular markers. Therefore, we decided to adopt mechanical loading in such a manner that cells were subjected to mechanical stretch for 2 consecutive hours and 6 consecutive hours. Tanabe showed that PPAR-γ1 and PPAR-γ2 could be downregulated only when mechanical stretch was applied in the late phase of adipogenic induction (30–45 h) (9), which is consistent with our mechanical stretch strategy.

Here, this loading protocol was effective in upregulating Runx2 and inhibiting Pref-1 and PPAR-γ in adipogenic medium; on the other hand, expression levels of these three markers did not demonstrate significant change in cells in normal medium. Runx2 is a transcription factor essential for osteoblast differentiation and upregulation early in the osteoblastic differentiation process. Pref-1, also known as DLK-1, has been considered to be a marker of undifferentiated preadipocytes, with mRNA expression reducing to low levels very rapidly as differentiation begins. There is evidence suggesting that preadipocytes are responsive to differentiation signals from the extracellular environment, through expression of plasma membrane protein Pref-1 that inhibits adipogenesis and

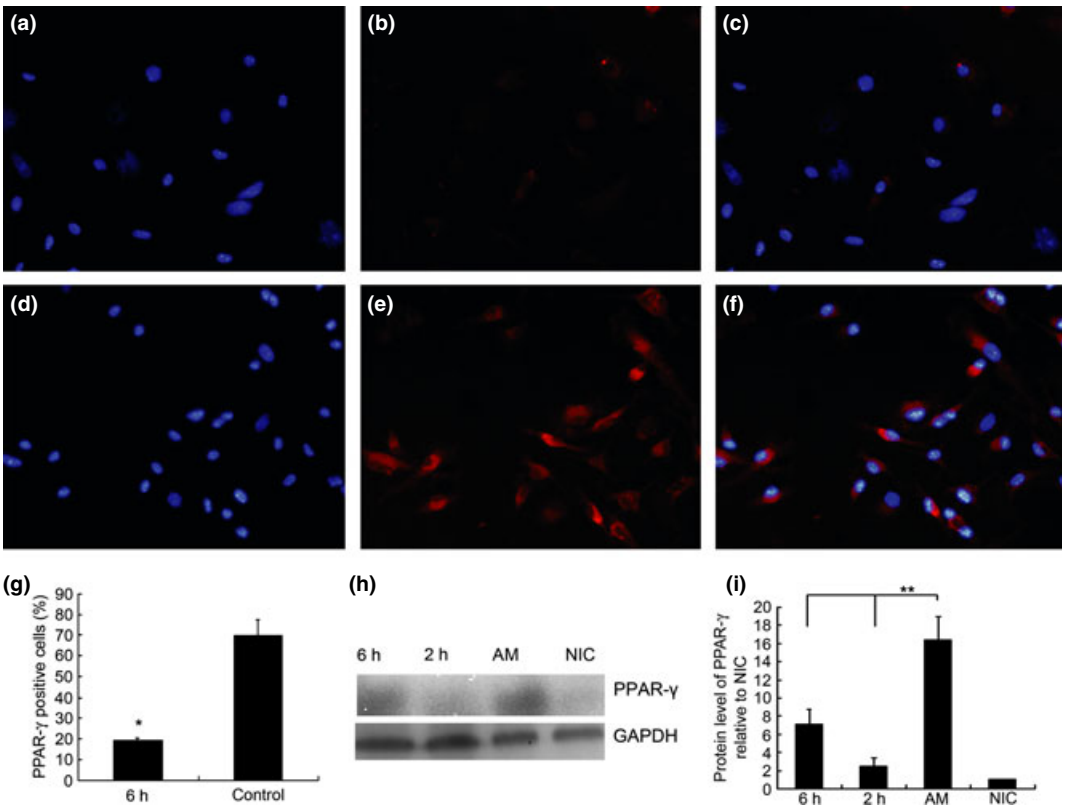

Figure 4. Effects of mechanical stretch on the PPAR-γ protein expression. Protein level was measured by immunofluorescence staining (a–g) and western blotting (h, i). Images (a–c) show immunofluorescence of PPAR-γ in cells kept in adipogenic medium for 72 h then mechanically loaded for 6 h, while images (d–e) show PPAR-γ staining in control groups only kept in adipogenic medium with no mechanical stretch loaded. Images (a) and (d) are DAPI staining of ASC, images (b) and (e) represent PPAR-γ staining, and (c) and (f) are merged images. PPAR-γ positive cells reduced from 69% to 19% by 6-h mechanical loading (g). Images H and I show western blotting results of PPAR-γ protein level. Six hours and 2 h mechanical stretch significantly reduced protein level of PPAR-γ compared to cells kept in adipogenic medium without mechanical stretch. ASCs kept in normal medium did not express PPAR-γ protein. Expression of each protein was normalized to that of GAPDH and noted as fold levels of protein NIC. Graphs are representative of three independent experiments. Values expressed as mean ± SEM; * $P < 0.05$; ** $P < 0.01$. AM, adipogenic medium group; NIC, the non-induced control group.

terminal adipocyte differentiation (18,19). Adipocyte differentiation *in vitro* is inhibited by Pref-1, but activated by the transcription factor, PPAR-γ. PPAR-γ has been examined in differentiated MSCs activating transcription of its target genes in adipogenic pathways. Sen *et al.* (17) showed that wild-type murine embryonic stem cells expressed PPAR-γ at high levels 4 days after adipogenic differentiation is induced. Thus, we examined PPAR-γ on the 4th day after adipogenic induction and 3rd day after mechanical stretch. Trends of mRNA and protein expression levels of Runx2, and PPAR-γ and mRNA level of Pref-1 indicated that mechanical stress inhibited adipogenesis and stimulated osteogenesis of ASCs in adipogenic medium.

Ability of cyclic stretch to inhibit adipogenic differentiation has previously been demonstrated in preadipocyte cell lines, bone marrow stromal cells and human umbilical cord perivascular cells (HUCPVC). Tanabe *et al.* (9) have demonstrated that cyclic stretch significantly reduced PPAR-γ expression, accumulation of triglyceride droplets and induction of glycerol-3-phosphate dehydrogenase in a 3T3-L1 preadipocyte cell line and David *et al.* (10) reported that cyclic stretching lead to downregulation of PPAR expression and upregulation of Runx2 in bone marrow cells, favouring osteogenesis over adipogenic differentiation. Turner *et al.* (11) further confirmed that exposure of HCUPVS to equibiaxial cyclic stretch (10%, 0.5 Hz) in the presence of adipogenic medium increased Smad2 phosphorylation compared to those in static samples and inhibited expression of adipocyte markers.

We also found that expression of adipogenic markers (PPAR-γ and Pref-1) and the osteogenic marker Runx2, was unchanged compared to static controls in response to cyclic stretch in normal medium without induction. In previous studies, differentiation markers only changed when

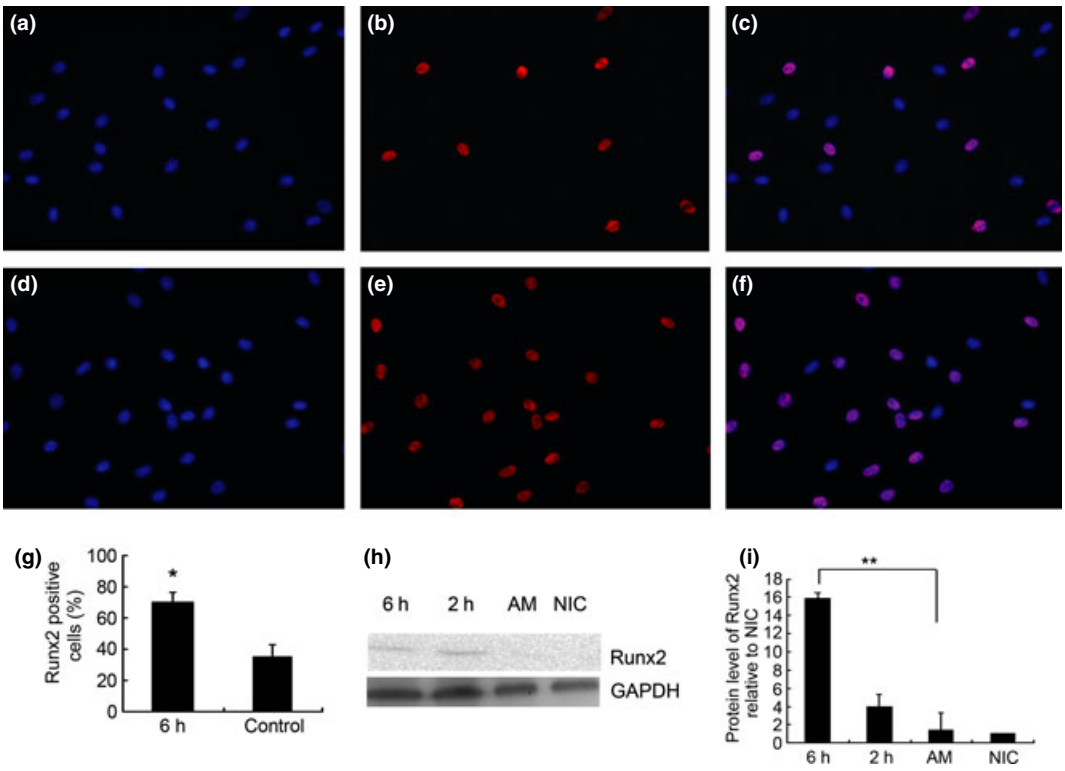

Figure 5. Effects of mechanical stretch on the Runx2 protein expression. Protein level was measured by immunofluorescence staining (a–g) and western blotting (h, i). Images (a–c) show the immunofluorescence staining of PPAR-γ in control groups retained in adipogenic medium only, with no loaded mechanical stretch, while images (d–e) show PPAR-γ in cells maintained in adipogenic medium for 72 h then mechanically loaded for 6 h afterwards. Images (a) and (d) are DAPI staining of ASC, images (b) and (e) represent Runx2 staining and (c) and (f) are merged images. Mechanical stretch significantly increased protein level of Runx2 (g). Images (h) and (i) show western blotting of PPAR-γ protein level. Six hours' mechanical stretch significantly increased protein level of Runx2 4-fold compared to cells maintained in adipogenic medium without mechanical stretch (h, i). ASCs kept in normal medium and adipogenic medium without mechanically loaded did not express Runx2 protein (h). Expression of each protein was normalized to that of GAPDH protein and noted as fold changes of protein level of NIC. Graphs representative of three independent experiments. Values expressed as mean ± SEM; $*P < 0.05$;$**P < 0.01$. AM, adipogenic medium group; NIC, non-induced control group.

mechanical loading conditions were applied during the entire adipogenic induction period (9,17) and in our previous studies, cyclic mechanical stress improved osteogenesis of ASCs under osteogenic-induced conditions (8). Data reported here show that Runx2 is markedly upregulated after mechanical loading with absence of osteogenic medium, and when cells had adipogenic stimulation. In fact, presence of adipogenic induction was required for upregulation of Runx2 as no significant increase was observed with standard medium. We hypothesize that induction initiated the whole differentiation process while mechanical loading could only change the fate of cell differentiation in the presence of induction medium. A further interesting result was that mechanical tension could upregulate osteogenic differentiation and downregulate adipogenic differentiation in an antagonistic way. In our previous studies, we found that osteoblasts and adipocytes share a common predecessor, the osteo-adipo progenitor cell (OAP), in murine adipose stromal cells (4); now we have demonstrated existence of OAPs in a further arena. Luu *et al.* (3) found that key osteogenic and adipogenic factors shifted in the biological balance, with increase in expression level of Runx2 and significant reduction in level of PPAR-γ. Considering that a reciprocal relationship between emergence of adipocytic and osteoblastic lineages exists, factors that drive differentiation towards one cell fate, suppress the other.

A range of mechanosensor molecules such as mechanically gated ion channels or membrane-integrated growth factor receptors has been proposed to transduce mechanical forces into intracellular signals (20,21). The ERK/-MAPK pathway is regarded as a key signalling convergence point that regulates mechanical force-induced upregulation of osteogenic differentiation of mesenchymal stem cells, during distraction osteogenesis (22,23). Prusty *et al.* (24) have suggested that stimulation

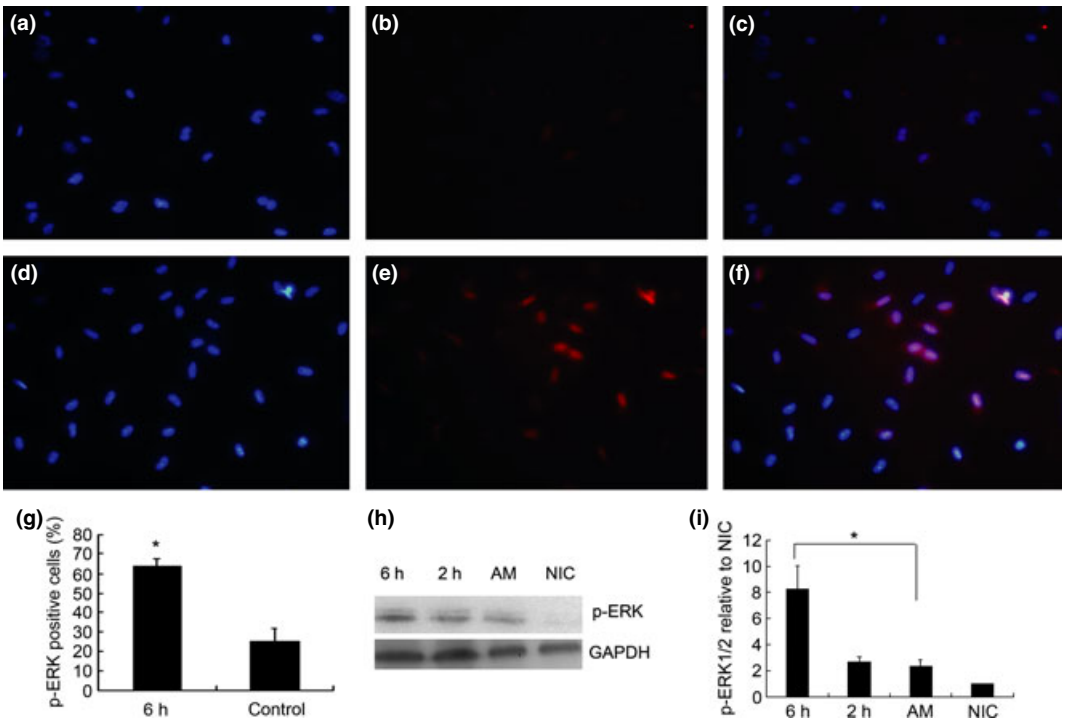

Figure 6. Effects of mechanical stretch on p-ERK protein expression. Protein level was measured by immunofluorescence staining (a–g) and western blotting (h, i). Images (a–c) show immunofluorescence of p-ERK in cells kept in adipogenic medium for 72 h then mechanically loaded for 6 h, while images (d–e) show p-ERK staining in control groups kept in adipogenic medium only, without mechanical stretch loading. Images (a) and (d) are DAPI staining of ASC, images (b) and (e) represent p-ERK staining, and (c) and (f) are merged images. Phospho-ERK positive cells were significantly augmented 3-fold by 6 h mechanical loading and trans-located to nuclei (f, g). Images (h) and (i) show western blotting of phosphor ERK protein level. Six hours mechanical stretch significantly increased protein level of p-ERK 3-fold compared to cells maintained in adipogenic medium only with no mechanical stretching. ASCs kept in normal medium did not express phospho-ERK protein. Expression of each protein normalized to that of GAPDH and noted by fold difference of protein level of NIC. Graphs representative of three independent experiments. Values expressed as mean ± SEM; $*P < 0.05$. AM, adipogenic medium group; NIC, non-induced control group.

of ERK/MAPK may have an opposing effect on the process of adipogenesis, depending on time of activation during differentiation. Moreover, only the inhibitory role of the ERK/MAPK pathway in adipocyte differentiation of 3T3-L1 cells was elicited in response to cyclic stretch, suggesting that prolonged or continuous activation of ERK/MAPK acts in an inhibitory manner in the context of adipocyte differentiation (25,26). In our study, ERK1/2 was activated by mechanical loading over longer duration, and phosphorylated ERK1/2 translocated to the nucleus, which was in accordance with results that have demonstrated the function of ERK in mechanical force-induced osteogenesis.

In conclusion, conversion of ASCs into adipocytes driven by adipogenic conditions can be inhibited by mechanical signals that also allow osteoblastic lineage selection, and ERK1/2 activation may be involved in this mechanical stress-induced trans-differentiation. Mechanical stress may function as a pivotal regulation factor in reciprocal relationships of osteogenic and adipogenic differentiation.

Disclosures

The authors indicate no potential conflict of interest.

Acknowledgements

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