

Mechanical Control of Mesenchymal Stem Cell Adipogenesis

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Abstract

Increased Mesenchymal Stem Cell (MSC) commitment and differentiation into adipocytes contributes to obesity. Other than dietary and biochemical factors, recent studies have begun to explore the role of mechanical signals in controlling MSC adipocytic commitment and differentiation. Several reported data suggest that by subjecting MSCs to certain mechanical stimuli, such as stretching, compression, and fluid shear, their adipogenesis could be inhibited or decreased. However, it is still very early to draw conclusions on the detailed mechanical regimens to optimally inhibit MSC adipogenesis and on the molecular pathways governing such adipogenesis-inhibitory mechanosensitive signaling. In this commentary, key data on the mechanical control of MSC adipogenesis and proposed molecular mechanisms will be highlighted and a future perspective in this new topic area will be provided.

Keywords: Mesenchymal Stem Cells (Mscs); Adipogenesis; Obesity; Mechanical Stimulation; Mechanotransduction

Mesenchymal stem cells (MSCs) differentiate into various types of cells that can form bone, cartilage, connective tissue, and fat. Obesity is characterized by increased adipocyte number and its hypertrophy with subsequent increase in adipose (fat) tissue formation. The increase in MSC commitment and differentiation into adipose cells, or adipogenesis, contributes to obesity. Therefore, understanding the extracellular factors that could promote or prohibit MSC adipogenesis may have an impact on how to deal with obesity. Studies have been performed to identify the effects of dietary biochemical factors on obesity using in vitro adipogenesis cell model and in vivo animal studies [1]. Recently, an unconventional non-biochemical approach has been exploited that tests how mechanical stimuli affect cellular commitment and differentiation into adipogenesis [2]. Mechanical factors that could affect the adipogenesis of MSCs or adipose precursor cells include cell stretching, compression, and fluid shear. Specific regimens of these mechanical stimuli could decrease or inhibit the adipocytic commitment and differentiation of these cells. However, molecular mechanisms relevant to disrupted adipogenesis under mechanical stimuli are not fully known. At the moment, a complete picture on the mechanical environments to downregulate cellular adipogenesis and mechanistic pathways governing such inhibition is yet to be fully revealed. This commentary will highlight key data on the mechanical control of MSC adipogenesis reported so far and propose a perspective on potential future research directions. This commentary is reminiscent of the book chapter recently published by our group [3]. Interested readers are referred to this book chapter for an extended review of the mechanical control of cellular adipogenesis.

It is notable that studies utilizing mechanical cell stimulations have not much focused on the MSC adipogenesis as a single research theme. Studies aimed at regenerative medicine purposes have used various mechanical stimulations to better support in vitro tissue engineering of mechanically functional tissues such as bone. Interestingly, it has been reported that inducing MSC and adipose stem cell fate decision into musculoskeletal lineages by mechanical stimuli may result in the reduction of stem cell commitment into adipogenesis [4,5]. Importantly, cells in adipose tissues are also physiologically exposed to compound mechanical cues (tensile, compressive, and shear) that result from bodyweight loads and weight-bearing, and it is becoming recognized that adipocytic cells and their precursors can be mechanically sensitive and responsive [2]. It is therefore probable that the process of cellular

evolution from MSCs and precursor cells into differentiated adipocytes may be influenced by extracellular mechanical signals. Key studies reporting the control of cellular adipogenesis by mechanical cell stretch, compression, and flow shear stress will now be highlighted.

The use of mechanical cell stretch to direct MSC fate toward osteogenesis has been widely adopted. In triggering osteogenesis, stretch signals could induce additional or synergistic effects when used with osteogenic differentiation media or hormones/cytokines such as bone morphogenetic proteins (see more details in our review [6]). In some cases, enhanced MSC osteogenesis by stretch was found to be associated with decreased adipogenesis. For instance, cyclic cell stretching of adipose-derived MSCs stimulated their osteogenesis while inhibiting adipogenesis, in which the trans-differentiation effect was attributed to the stretch upregulation of Extracellular Signal-Regulated Kinase (ERK) [4]. Also, cyclic stretching of C3H10T1/2 murine MSCs could overcome the adipogenic induction given by the adipogenic differentiation media. For instance, 2% cyclic strain increased Runx2 and osterix (markers of bone cell differentiation) but decreased peroxisome proliferator-activated receptor γ (PPAR γ) and adiponectin (adipogenic transcription factor/marker) in MSCs, in which the stretch induction of β -catenin nuclear translocation was proposed as a regulatory mechanism [7]. The degree to which MSC adipogenesis is inhibited by cyclic cell stretching may depend on the magnitude of the strain and frequency and the rest period between each applied load. For instance, in a study using low strain but high frequency ($<10 \mu$ strain, 90 Hz) and high strain low frequency (20,000 μ strain, 0.17 Hz), both stimuli could reduce MSC adipogenesis when at least a 1 h refractory period between bouts was given [8]. However, given the limited number of systematic studies on this topic, it is difficult to draw a conclusion regarding the stretch regimens to optimally inhibit MSC adipogenesis.

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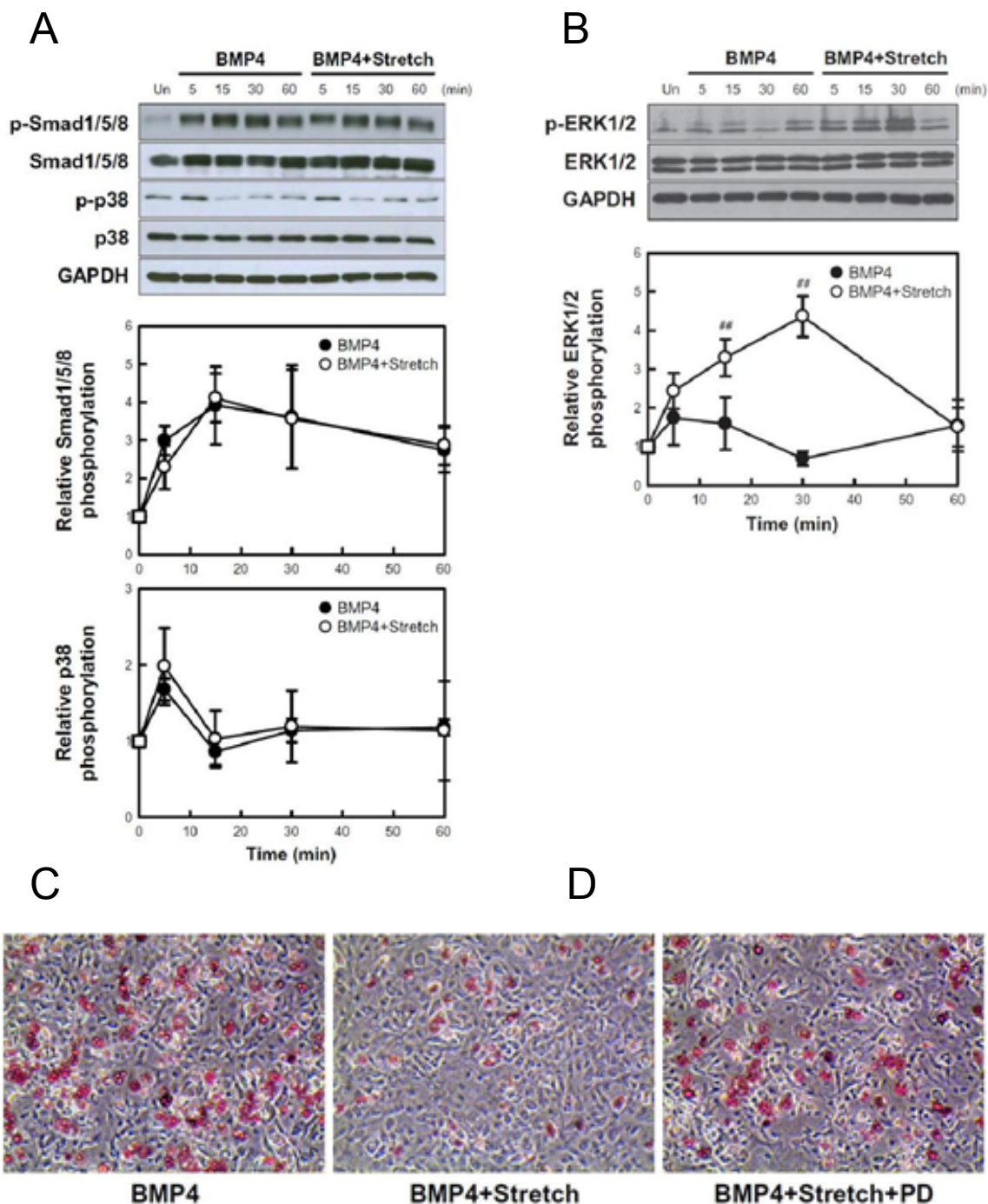


Figure 1: Cyclic mechanical stretch suppresses MSC adipogenesis under BMP4 pre-treatment followed by adipogenic media exposure, and the suppression is potentially achieved via ERK signaling. (A) BMP4 signaling through Smad and p38 in MSCs was not affected by cyclic mechanical stretching. (B) ERK1/2 was significantly upregulated in MSCs by cyclic mechanical stretch relative to BMP4 treatment alone sample (## $p < 0.01$ at the same time point). (C, D) The effect of cyclic stretch suppression of BMP4-mediated MSC adipogenesis was decreased when ERK is blocked by PD98059 (PD). # $p < 0.05$, ## $p < 0.01$ compared with BMP4; $\psi p < 0.05$ compared with BMP4 plus stretch. Reprinted with permission from Elsevier [9]

MSC developmental stage may also affect the effectiveness of mechanical stretch in its blocking adipogenesis. In our own study, it was shown for the first time that cyclic stretch (10% strain, 0.25 Hz) applied during the MSC commitment stage (before the adipogenic induction period) could suppress MSC adipogenic differentiation [9]. If C3H10T1/2 MSCs were stretched during the BMP4 pre-treatment

period (commitment period), the subsequent response of the cells to adipogenic hormonal inducers was suppressed. We further observed that this stretch inhibition of adipogenesis could be mediated by ERK1/2 activation but not through the downregulation of Smad or p38 pathway (Figure 1). Note that in previous studies stretching was applied while cells were exposed to the adipogenic induction media [4,7].

One recent study proposed that focal adhesion-cytoskeletal signaling, such as the upregulation of mTORC2 by cell stretch, may play a role in cyclic stretch induction of MSC osteogenesis over adipogenesis [10]. In addition to ERK, β -catenin, and mTORC2 as referenced above, an apparent trans-differentiation mechanism has been tested, that is, cell stretch could increase Runx2 at the expense of decreasing PPAR γ , key osteogenic and adipogenic transcription factors, respectively [5]. In the same study, the adipocytic induction by rosiglitazone, an established PPAR γ agonist, could be partially overcome by stretch, as demonstrated by favored osteogenesis.

The stretch studies described above indicate a strong potential of cyclic stretch to suppress MSC adipogenesis. It is notable that noncyclic stretch, which applies elongation then maintains the strain for a given period of time, may induce an opposite effect. This was reported for 3T3-L1 preadipocytic cell line cells subjected to 12-20% noncyclic stretching motion [11,12]. The increase in the adipogenesis of 3T3-L1 cells by noncyclic stretching was attributed to the increase in RhoA kinase (ROCK) [11] and Mitogen-Activated Protein Kinase (MAPK) signaling [12], respectively. In the presence of pharmacological inhibitors of ROCK and MAPK, the increase in adipogenesis by noncyclic stretch was impaired. The observation that noncyclic stretch actually increases adipogenesis, although it was reported for 3T3-L1 pre-adipocytes only, may suggest in combination with the above conclusion on the cyclic stretch inhibition of MSC adipogenesis an implication on the exercise control of obesity. Only dynamic exercises (walking, running, etc.) potentially corresponding to the cyclic stretching of the cells may have an effect to reduce fat synthesis and deposition. However, it is noteworthy that the precise correlation between the macro-motion of the body and strains to which cells in the body (including MSCs) are exposed is not fully known (see below for more discussion).

The other mechanical stimulation mode, such as compression, has not been utilized yet for testing MSC adipogenesis. One report used SGBS (Simpson-Golabi-Behmel Syndrome) preadipocytic cells derived from human fat tissue and demonstrated that compression may decrease their adipocytic differentiation [13]. The application of the compressive force at 226 Pa for 12 h before the adipogenic induction could successfully inhibit the adipogenesis of SGBS cells. However, applying compressive force after the adipogenic induction period did not produce a significant effect. This study further revealed that in the presence of cyclooxygenase-2 (COX-2) inhibitor the compression inhibition of adipogenesis was lost. This study on cell compression, though it is not for MSCs, suggests a similarity with the cyclic stretch inhibition of adipogenesis described above. Also, the blockage of SGBS adipogenesis by the compression applied before the adipocytic induction period may be analogous with the cyclic stretch suppression of MSC adipogenesis when applied during the commitment period [9]. Since most of the cell compression studies have had a goal to improve the chondrogenesis of MSCs, more studies on the adipogenic lineage at varying compression regimens and for different types of adipose precursor cells are needed to provide a complete comparison.

A positive role of fluid flow-induced shear stress stimulation in facilitating MSC osteogenesis has been relatively well established (see our review [14]). Cells embedded in bone are exposed to shear stress from interstitial flow through lacunar-canalicular channels. Considering that this flow stimulation positively regulates the osteogenic activity of bone cells, it has been proposed that such a flow may also affect the ability of MSCs to differentiate toward bone cells. On the other hand, there has not been a study solely focusing on the effect of fluid flow on MSC adipogenesis. One study demonstrated that MSC differentiation into multiple lineages, osteogenesis, chondrogenesis,

and adipogenesis, may be influenced by flow shear [15]. MSCs exposed to fluid flow actually showed upregulation in all three transcription factors of Runx2, Sox9, and PPAR γ , each governing the osteogenic, chondrogenic, and adipogenic fate. When cytoskeletal formation was disrupted by cytochalasin (actin inhibitor), Y27632 (ROCK inhibitor), and blebbistatin (Myosin II inhibitor), the increase in Sox9 and PPAR γ by fluid flow disappeared, suggesting the role of cytoskeletal structure in flow shear control of MSC fate. More recently, it was proposed that fluid flow has the potential to decrease MSC adipogenesis [16], which effect is similar to the cyclic cell stretch and compression data. This study exposed MSCs to fluid shear within a multi-shear microfluidic channel. With increasing shear stress level MSCs exhibited greater expression of Yes-Associated Proteins (YAP), which in turn influenced MSC fate decision, i.e., decrease in adipogenesis, increase in osteogenesis, and dedifferentiation for chondrogenesis.

It is clear based on these data that mechanical stimuli such as stretch, compression, and fluid shear have a substantial potential to influence MSC adipogenesis. In most cases (other than noncyclic stretching), these mechanical cell stimulations were found to be able to decrease the commitment and differentiation of MSCs toward adipogenesis. Therefore, determining optimal mechanical regimens to maximally suppress the MSC adipocytic commitment and differentiation may propose new means to deal with obesity and related health concerns such as metabolic syndrome.

In order for the research data obtained so far to be more meaningful, several aspects need to be clarified. First, correlations must be established between the mechanical regimens applied in the in vitro mechanical cell stimulation studies and the motion of the human body arising from various motions/exercises. Depending on the location of the cells (MSCs and adipocytic precursor cells) within the body, the exact strain and strain rate to which the cells are exposed due to the body motion should be quantified. Then the data from in vitro mechanical cell stimulations may be used to potentially predict the effects of exercises on obesity. Such correlation for MSCs located in adipose tissue and bone marrow is not yet available. Second, systematic studies with varying mechanical regimens are required to determine the mechanical conditions optimal for maximizing MSC adipogenesis inhibition, considering the limited amount of accumulated data especially for the compression and fluid flow. Furthermore, one may have to consider that the mechanical stimulations of stretch, compression, and flow shear often occur in vivo in a combined manner. For example, bending of the long bone induces stretching on one side and compressive force on the other side. Fluid flow through interstitial channels within a tissue is commonly a result of stretch or compression of the tissue. Thus, experimental design to effectively exclude or combine the effects of the independent mechanical cues is required. Third, while various studies have proposed several molecular mechanisms of the mechanical inhibition of MSC adipogenesis, such as ERK, β -catenin, mTORC2, COX-2, YAP, etc., it is premature to select the governing molecular mechanosensor. It is quite true that the mechanistic pathways responsible for MSC adipogenesis are not fully understood yet even for the static culture condition. We recently reported that focal adhesion kinase (FAK), one of the vital cascades of integrin-mediated focal cell adhesion, may play a regulatory role in MSC adipogenesis under static culture (Figure 2) [17]. If a molecular target could be identified, either for static culture or mechanical stimulation condition, a pharmaceutical approach may be taken to better treat obesity issues. For instance, sensitizing specific molecular mechanosensor may allow MSCs and adipocytic precursor cells to be highly mechano-responsive to the given mechanical stimulation. If so, in the best case scenario,

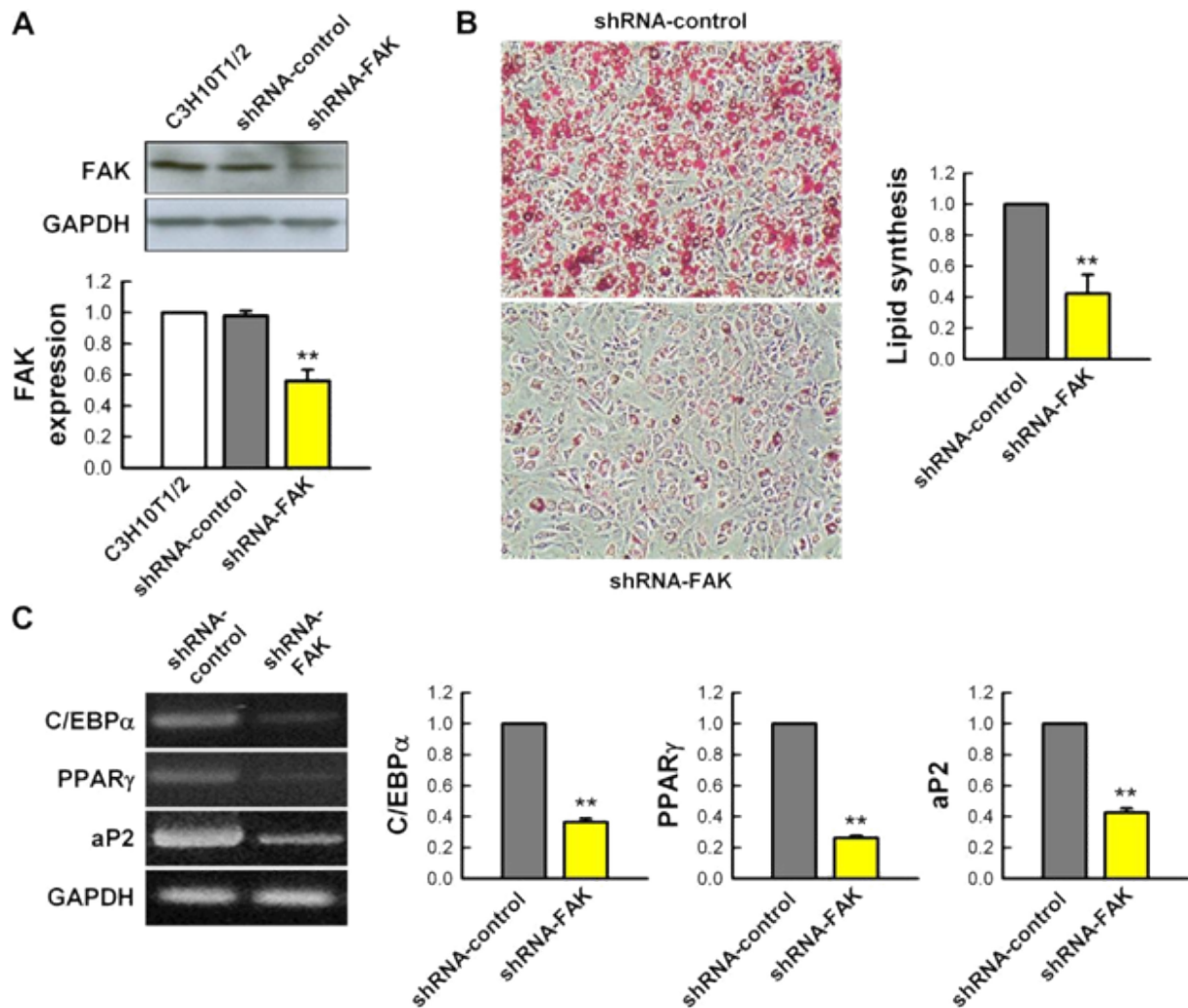


Figure 2: MSC adipogenesis under BMP4 pre-treatment followed by adipogenic media exposure is suppressed by FAK silencing. (A) FAK interference using small hairpin RNA (shRNA) confirmed by immunoblotting. (B, C) MSC adipogenesis under BMP4 pre-treatment followed by adipogenic media exposure was suppressed by FAK shRNA, as assessed by lipid synthesis and adipogenic genes. ** $p < 0.01$ compared with shRNA-control. Reprinted with permission from Elsevier [17].

one may not deposit fat tissue even under mild everyday activities without doing hardcore exercises. This mechanical approach differs considerably from conventional dietary and biochemical approaches, which may shed a new insight into how to control cellular adipogenesis and thus obesity. Additionally, appropriate mechanical stimulation may serve to augment dietary and biochemical signaling inputs, making them more effective in controlling obesity.

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