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Published in:
Stem Cell Research

DOI:
[10.1016/j.scr.2018.03.010](https://doi.org/10.1016/j.scr.2018.03.010)

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Chen, L., Hu, H., Qiu, W., Shi, K., & Kassem, M. (2018). Actin depolymerization enhances adipogenic differentiation in human stromal stem cells. *Stem Cell Research*, 29, 76-83.
<https://doi.org/10.1016/j.scr.2018.03.010>



Actin depolymerization enhances adipogenic differentiation in human stromal stem cells



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ARTICLE INFO

Article history:

Received 6 September 2017

Received in revised form 15 March 2018

Accepted 15 March 2018

Available online 18 March 2018

Keywords:

Actin cytoskeleton

Actin depolymerizing factors

Adipocyte differentiation

Human stromal stem cells

ABSTRACT

Human stromal stem cells (hMSCs) differentiate into adipocytes that play a role in skeletal tissue homeostasis and whole body energy metabolism. During adipocyte differentiation, hMSCs exhibit significant changes in cell morphology suggesting changes in cytoskeletal organization. Here, we examined the effect of direct modulation of actin microfilament dynamics on adipocyte differentiation. Stabilizing actin filaments in hMSCs by siRNA-mediated knock down of the two main actin depolymerizing factors (ADFs): Cofilin 1 (CFL1) and Destrin (DSTN) or treating the cells by Phalloidin reduced adipocyte differentiation as evidenced by decreased number of mature adipocytes and decreased adipocyte specific gene expression (ADIPOQ, LPL, PPARG, FABP4). In contrast, disruption of actin cytoskeleton by Cytochalasin D enhanced adipocyte differentiation. Follow up studies revealed that the effects of CFL1 on adipocyte differentiation depended on the activity of LIM domain kinase 1 (LIMK1) which is the major upstream kinase of CFL1. Inhibiting LIMK by its specific chemical inhibitor LIMKi inhibited the phosphorylation of CFL1 and actin polymerization, and enhanced the adipocyte differentiation. Moreover, treating hMSCs by Cytochalasin D inhibited ERK and Smad2 signaling and this was associated with enhanced adipocyte differentiation. On the other hand, Phalloidin enhanced ERK and Smad2 signaling, but inhibited adipocyte differentiation which was rescued by ERK specific chemical inhibitor U0126. Our data provide a link between restructuring of hMSCs cytoskeleton and hMSCs lineage commitment and differentiation.

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1. Introduction

The cytoskeleton is a system of intracellular filaments crucial for cell shape, adhesion, growth, division and motility (Stossel, 1993; Wickstead and Gull, 2011). As one of the major component of cytoskeleton, actin filaments form a highly dynamic network composed of actin polymers and a large variety of associated proteins (Schmidt and Hall, 1998). The actin proteins exist within cells in either globular/monomer (G-actin) or filamentous (F-actin) form and thus in dynamic transitions between depolymerization and polymerization status (Ono, 2007). Actin depolymerization factor (ADF) cofilin is a family of actin binding proteins with actin filaments depolymerization function (Bamburg et al., 1980; Nishida et al., 1984). In mammals, the cofilin family consists of three highly similar paralogs: Cofilin-1 (CFL1, non-muscle cofilin, n-cofilin), Cofilin-2 (CFL2, muscle cofilin, m-cofilin) and destrin (DSTN). The activity of cofilins is regulated by phosphorylation,

polyphosphoinositide interaction, pH and interaction with other actin binding proteins (Van Troys et al., 2008).

Human bone marrow-derived stromal (Skeletal) stem cells (hMSCs) are a group of clonogenic and multipotent cells capable of differentiation into mesoderm-type cells e.g. osteoblast and adipocyte (Abdallah and Kassem, 2008). During lineage specific differentiation, hMSCs exhibit significant changes in morphology and actin cytoskeletal organization (McBeath et al., 2004; Treiser et al., 2010; Yourek et al., 2007). Previous studies have suggested that RhoA-ROCK (Ras homolog gene family member A-Rho-associated protein kinase) signaling mediates changes in cell shape and cytoskeletal tension regulating hMSC lineage commitment (McBeath et al., 2004). A spheroidal morphology associated with a dispersed actin cytoskeleton with few focal adhesions encourages MSC differentiate into adipocytes whereas a stiff, spread actin cytoskeleton with greater numbers of focal adhesions drives MSC differentiate into osteoblasts (Mathieu and Lobo, 2012). The changes in cell shape are caused by cytoskeletal changes due to actin synthesis and reorganization (Antras et al., 1989; Spiegelman and Farmer, 1982), and thus modification by mechanical forces or regulation of relative kinases that change actin cytoskeletal dynamics, can regulate cell differentiation (Arnsdorf et al., 2009; Kanzaki and Pessin, 2001;

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McBeath et al., 2004; Noguchi et al., 2007). However, this approach has not been widely utilized in differentiation studies of hMSCs.

We have previously reported that interfering with actin assembly dynamics by knocking down cofilin-1 in hMSCs increased polymerized actin that promoted osteoblast cell differentiation through a mechanism of enhancing focal adhesion kinase (FAK), p38 and c-Jun N-terminal kinase (JNK) signaling (Chen et al., 2015). In the present study, we examined the effects of interfering in the actin cytoskeletal dynamics on the regulation of adipocyte differentiation of hMSCs.

2. Materials and methods

2.1. Cell culture and adipocyte differentiation of hMSCs

Primary hMSCs were isolated from human bone marrow aspirate or adipocyte as described (Stenderup et al., 2004). As a model for primary hMSCs, we employed the hMSCs-TERT cell (hMSCs here after) created in our laboratory by overexpression of human telomerase reverse transcriptase (hTERT) gene (Simonsen et al., 2002). Cells were cultured in Minimal Essential Media (MEM) without Phenol red and L-glutamine, supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM Glutamax, 100 units/ml penicillin, and 100 µg/ml streptomycin (Gibco-Invitrogen, Carlsbad, CA). For adipocyte differentiation, cells were induced by adipogenic induction medium containing 10% horse serum (Sigma), 100 nM dexamethasone (Sigma), 450 µM IBMX (1-methyl-3-isobutylxanthine, Sigma), 1 µM BRL (Cayman Chemical), 3 µg/ml insulin (Sigma). Media were changed every two days. For chemical treatment experiment, before each medium change, the cells were pre-treated with Cytochalasin D (Sigma, C8273) (1–20 µM) for 1 h or Phalloidin (Sigma, P2141) (0–3 µM) for 3 h or DMSO as control. After treatment, cells were washed once and incubated in adipogenic differentiation medium. In some experiments, 1 µM LIM domain kinase inhibitor (LIMKi) (Calbiochem) or 10 µM and 25 µM ERK inhibitor U0126 (Cell Signaling, #9903) was used continuously with adipocyte induction medium during adipocyte differentiation, Oil red O staining for lipid droplets formation in mature adipocytes was performed as described (Abdallah et al., 2005).

2.2. Cell transfection

Small interfering RNA (siRNA) targeting DSTN (siR-DSTN), CFL1 (siR-CFL1) as well as non-targeting siRNA (siRNA negative control, siR-Ctrl) were purchased from Ambion (Life Technology Inc.). siRNAs were transfected into hMSCs-TERT at a final concentration of 10 nM by Lipofectamine2000 (Invitrogen) according to the manufacturer's instructions. 2–3 siRNAs for each gene were ordered and tested in the study to confirm the result.

2.3. Western blot analysis

Western blot analysis was performed as described previously (Chen et al., 2015). Antibodies (total or phosphor) specific for CFL1, LIMK1, Smad2, RhoA, Akt, JNK, p38, Smad1/5/8 were obtained from Cell Signaling Technology; antibodies for total and phosphor-ERK were purchased from Santa Cruz; antibodies for DSTN, α -tubulin and actin were bought from Sigma. All antibodies used at 1:1000 dilutions except α -tubulin and actin antibodies (1:5000) in this study.

2.4. G-actin/F-actin assay

G-actin and F-actin in cells were quantitated by G-actin/F-actin in vivo assay kit as manufacturer's instructions (Cytoskeleton® Inc.). Cells were harvested by scraping in lysis and F-actin stabilization buffer. The homogenates were incubated at 37 °C for 10 min, transferred to a pre-warmed (37 °C) ultracentrifuge (SORVALL/Thermo Scientific) and spun at 100,000g for 1 h at 37 °C to separate the globular (G)-actin

(supernatant) and filamentous (F)-actin fractions (pellet). The pellets were re-suspended in ice-cooled depolymerizing buffer. All samples were diluted with appropriate loading buffer for Western blot analysis. The blots were scanned and the protein bands were subjected to intensity quantification in ImageJ® software. Ratios of F-actin or G-actin in cells were calculated according to the density.

2.5. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR)

For gene expression, total RNA was extracted with TriZol reagent (Invitrogen), and cDNA was prepared using RevertAid H Minus First Strand cDNA Synthesis kit (Fermentas). The PCR products were visualized in real-time using SYBR Green I Supermix (Bio-Rad) and an iCycle instrument (Bio-Rad) using standard curve protocols, normalized to beta-2-microglobulin (B2m). The quantitative data presented is an average of duplicate or triplicate per independent experiment.

2.6. Statistical analysis

Data are presented as mean \pm standard deviation (SD). Statistical testing was performed using Student's *t*-test to detect differences between groups. Differences were considered statistically significant at $P < .05$ (*) or $P < .01$ (**).

3. Results

3.1. Actin cytoskeleton is disassembled during adipocyte differentiation of hMSCs

The success of adipocyte differentiation of hMSCs was evidenced by the upregulation of both early and late adipocyte gene markers including peroxisome proliferator activated receptor gamma 2 (PPARG2), Adiponectin (ADIPOQ), lipoprotein lipase (LPL) and fatty acid binding protein 4 (FABP4) (Fig. 1A) during a 13-day in vitro differentiation protocol. As shown in Fig. 1A, Oil-red O staining revealed the formation of mature lipid-filled adipocytes that increased in number throughout differentiation period. By determining the G-actin/F-actin ratio, we observed that monomeric G-actin was increased from 59% to 76% and assembled F-actin decreased from 42% to 24% during the adipocyte differentiation (day 0 and day 13 values, respectively) (Fig. 1B).

Since actin cytoskeleton is regulated by RhoA/LIMK1/CFL1 axis (Maekawa et al., 1999), we examined their expression and activity during adipocyte differentiation (Fig. 1C). We observed that the ratio of phosphor-CFL1 (p-CFL1) to total CFL1 indicating accumulation of the inactive form of CFL1 lacking the ability to disassemble actin fibres, decreased during adipogenic induction mostly and this was caused by highly increased expression of total CFL1 as soon as adipogenic induction started (Fig. 1C). The total and phosphorylation level of LIMK1, as well as the expression of RhoA, which are the upstream kinases responsible for CFL1 phosphorylation, were also reduced during adipocyte differentiation (Fig. 1C). The expression of another actin depolymerisation factor DSTN was unchanged (Fig. 1C). The results suggest that the observed decreased in assembled actin during adipocyte differentiation was associated with changes in RhoA/LIMK1/CFL1 signaling pathway.

3.2. Modulation of actin polymerization regulates adipocyte differentiation of hMSCs

To corroborate the importance of actin assembly for adipocyte differentiation, we examined the effects of cytochalasin D (CytoD) or Phalloidin, which have been shown to disassemble or stabilize the actin cytoskeleton in hMSCs, respectively (Chen et al., 2015), on adipocyte differentiation of hMSCs. We observed that CytoD inhibited but Phalloidin increased cell viability during adipocyte differentiation (Fig. 2A). Disruption of actin assembly by CytoD increased the

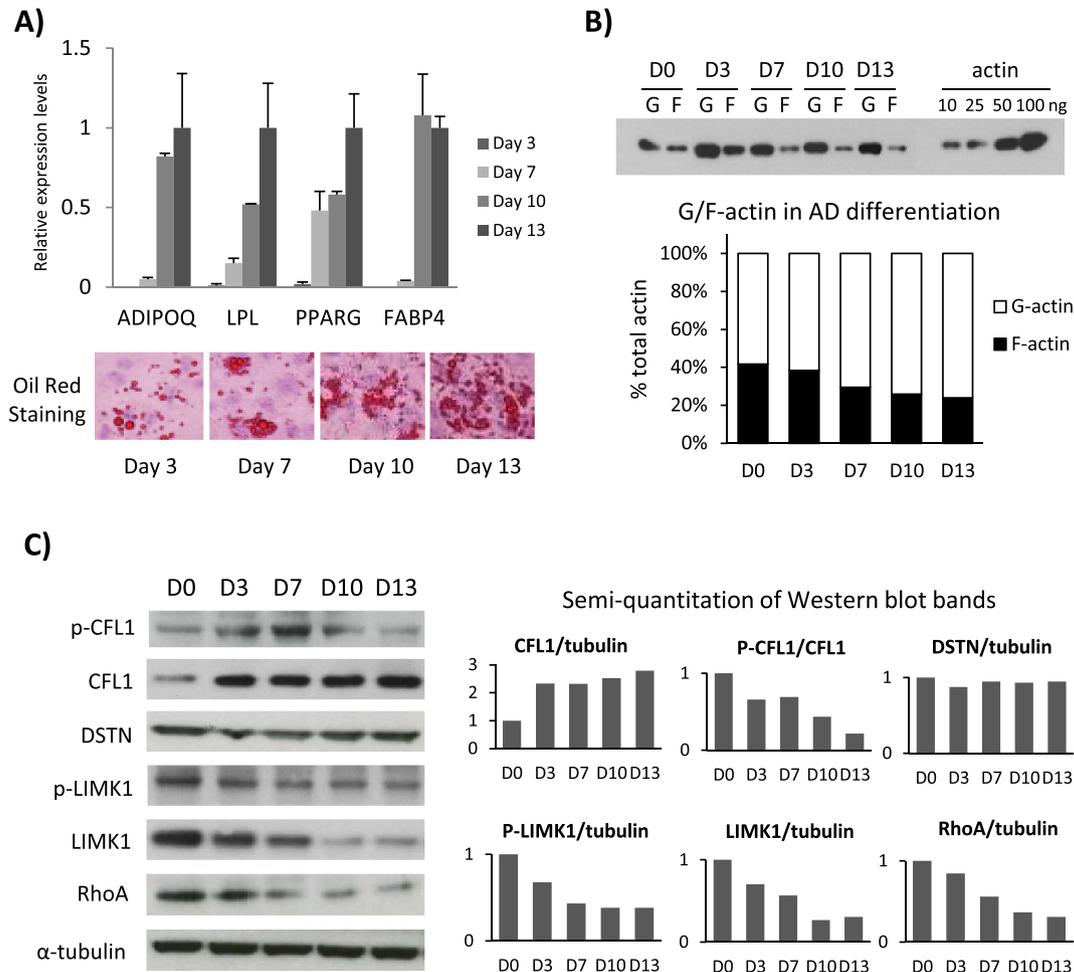


Fig. 1. Actin cytoskeleton is dissembled during adipocyte differentiation of hMSCs via RhoA/LIMK1/CFL1 axis. hMSCs were treated with adipogenic induction medium for the indicated days. Representative data were shown from three independent experiments. A. The expression of adipogenic markers were analyzed by qRT-PCR and normalized against B2M. The expression level at day 13 was set to 1. Data were presented as mean + S.D., $n = 3$ (upper). Mature adipocytes with lipid droplets were stained by Oil-red O staining (lower). B. G-actin and F-actin level were analyzed by G-actin/F-actin assay followed by quantitation of band density by ImageJ software. C. The expression and phosphorylation status of CFL1, LIMK1, RhoA and DSTN was analyzed by western blot. α -tubulin was used as loading control. Band density in the Western blot was semi-quantitated by ImageJ software.

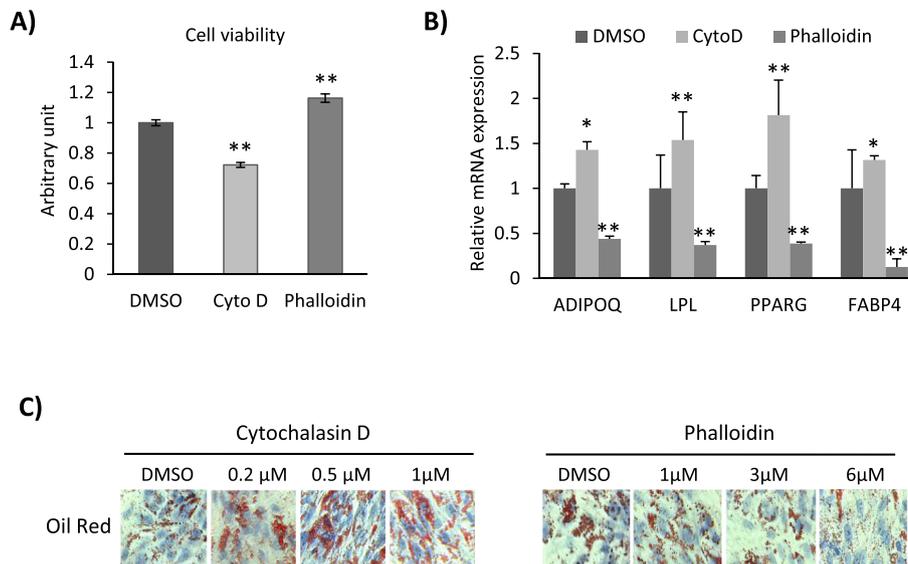


Fig. 2. Actin cytoskeleton dynamics affects adipocyte differentiation of hMSCs. hMSCs were treated with DMSO as control, 0.5 μ M Cytochalasin D (Cyto D), or 3 μ M Phalloidin as indicated in Material and Methods, underwent adipocyte differentiation for 10 days. Representative data were shown from three independent experiments. * $P < .05$, ** $P < .01$. A. Cell viability was measured by CellTiter-Blue reagent. Data were presented as mean \pm S.D., $n = 8$. B. The expression of adipogenic markers including ADIPOQ, LPL, PPARG and FABP4 was determined by qRT-PCR and normalized against B2M. The expression level with DMSO treatment was set to 1. Data were presented as mean + S.D., $n = 3$. C. The accumulation of lipid droplets in mature adipocytes was visualized by Oil-red O staining on Day 10 for Cyto D treatments and Day 13 for Phalloidin treatments.

expression of adipocyte gene markers (Fig. 2B) and increased the number of mature adipocytes (Fig. 2C, left). In contrast, stabilization of actin cytoskeleton by Phalloidin inhibited adipocyte differentiation evidenced by reduced expression of adipocytic gene markers (Fig. 2B) as well as reduced number of mature adipocytes (Fig. 2C, right). Similar results were also got from the culturing human primary bone marrow derived MSC and adipose-derived MSC (data not shown).

3.3. Prevention of actin disassembly by knocking down CFL1 and DSTN inhibits adipocyte differentiation of hMSCs

To further confirm the effects of actin dynamic status on adipocyte differentiation, we stabilized actin cytoskeleton by decreasing levels of CFL1 and DSTN using the specific siRNA (siR) and investigated the effects on adipocyte differentiation. siR-CFL1 and siR-DSTN resulted in >80% decrease in levels of CFL1 and DSTN (Fig. 3A) and significant changes in cell viability (Fig. 3B). Cells deficient in CFL1 or DSTN exhibited significant reduction in their adipocyte differentiation capacity (Fig. 3C).

3.4. Inhibition of LIMK1 increased adipocyte differentiation of hMSCs

LIMK1 phosphorylates CFL1 at Ser3 and thus inhibits its actin severing activity (Arber et al., 1998). We treated hMSCs with LIMK inhibitor (LIMKi) during adipocyte differentiation and as expected, CFL1 phosphorylation was inhibited (Fig. 4A). LIMKi treatment enhanced adipocyte differentiation and significantly increased the expression of

adipocyte gene markers CEBPA, FABP4 and ADIPOQ and to lesser degree of PPARG2 and LPL (Fig. 4C).

3.5. Actin assembly dynamics regulate adipocyte differentiation of hMSCs via ERK and Smad2 signaling

We investigated signaling pathways associated with changes in actin microfilament dynamics during adipocyte differentiation. Decreased assembled actin by CytoD treatment decreased Smad2 and ERK MAPK phosphorylation, while stabilized assembled actin by Phalloidin treatment exerted opposite effects (Fig. 5A). In addition, we treated the cells by specific ERK phosphorylation inhibitor U0126 (Fig. 5B) and we observed reversal of the inhibitory effects on adipocyte differentiation by Phalloidin (Fig. 5C and D). We did not detect significant changes of Akt, Smad1/5/8, p38 or JNK MAPKs signaling pathways upon treatment with CytoD or Phalloidin (data not shown).

4. Discussion

Changes in hMSCs cell morphology correlate with in vitro and in vivo differentiation to osteoblasts and adipocytes. In the current study, we demonstrated that adipocyte differentiation of hMSCs is associated with significant changes in actin filament structure with increased G-monomeric actin and decreased F-actin and that these changes were accompanied by changes in actin depolymerizing factor CFL1 which involves in upstream kinase LIMK1 and downstream ERK kinase.

Actin cytoskeleton undergoes cycles of actin assembly and disassembly in a balanced fashion between two states: a monomeric state

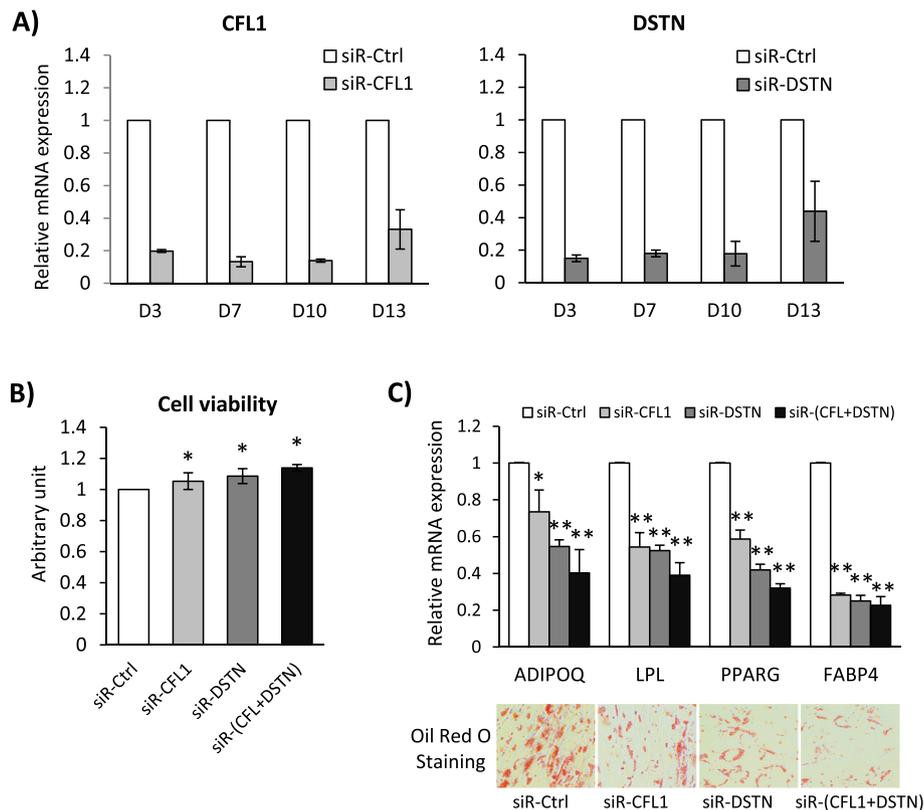


Fig. 3. Stabilization of actin cytoskeleton by knocking down CFL1 and DSTN inhibits adipogenesis of hMSCs. hMSCs were transfected with control non-targeting siRNA (siR-Ctrl) or siRNA targeting CFL1 (siR-CFL1), DSTN (siR-DSTN) or both and then treated with adipogenic induction medium for up to 13 days. Representative data were shown from three independent experiments. * $P < .05$, ** $P < .01$. A. Knocking down efficiency was determined by qRT-PCR and normalized against B2M at indicated day. The gene expression with siR-Ctrl was set to 1. Data were presented as mean \pm S.D., $n = 3$. B. Cell viability was measured by CellTiter-Blue reagent. Data were presented as mean \pm S.D., $n = 8$. C. The expression of adipogenic markers including ADIPOQ, LPL, PPARG and FABP4 was determined by qRT-PCR and normalized against B2M at day 10 (upper). The expression level with siR-Ctrl was set to 1. Data were presented as mean \pm S.D., $n = 3$. The accumulation of lipid droplets in mature adipocytes was visualized by Oil-red O staining at day 10 (Lower).

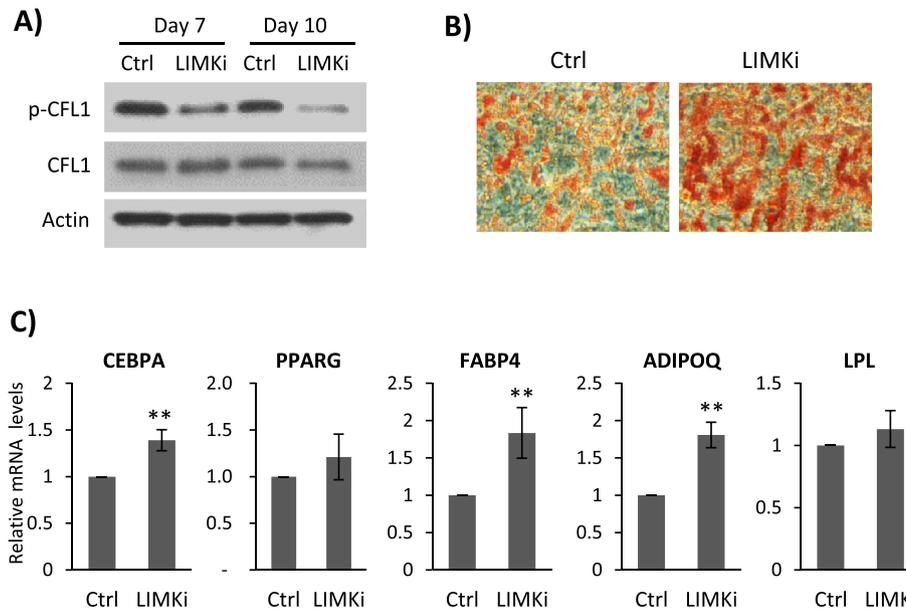


Fig. 4. Inhibition of LIMK1 increased adipocyte differentiation of hMSCs by activation of CFL1. hMSCs were treated with adipogenic induction medium supplemented with DMSO as control (Ctrl) or 1 μ M LIMK inhibitor (LIMKi) for 7 or 10 days continuously. Representative data was shown from three independent experiments. ** $P < .01$. A. The total and phosphor-CFL1 were detected by western blot. Actin was used as loading control. B. The accumulation of lipid droplets in mature adipocytes was visualized by Oil-red O staining at day 10. C. The expression of adipogenic markers including CEBPA, ADIPOQ, LPL, PPARG and FABP4 was determined by qRT-PCR and normalized against B_2M . Data were presented as mean \pm S.D., $n = 3$.

(globular, G-actin) and a fibrous state (filamentous, F-actin). We have observed that adipocyte differentiation of hMSCs is associated with increased in G-actin and these changes were the opposite of what we have previously reported during osteoblastic differentiation of hMSC (Chen et al., 2015). Since G-actin/F-actin is important determinant of cell shape, our findings corroborate previous studies showing that restructuring of cellular morphology during differentiation influences lineage-specific commitment (Carvalho et al., 1998; McBeath et al., 2004; Spiegelman and Ginty, 1983).

We observed significant changes in actin depolymerizing factors during hMSCs differentiation into adipocytes. CFL1 is ubiquitously expressed, actin depolymerizing factor that limits actin polymerization by binding (the non-phosphorylated form) to F-actin without capping and sever the F-actin into monomers free ends actin (G-actin) (Arber et al., 1998). CFL1 activity depends on serine 3 phosphorylation mediated by LIM kinase (LIMK), which in turn regulated by the Rho small GTPase and Rho-associated protein kinase (ROCK) (Maekawa et al., 1999). We observed that high levels of non-phosphorylated CFL1, and low level of LIMK1 and RhoA enhance adipocyte differentiation and lead to cellular enrichment of depolymerized actin.

We have previously reported that stabilizing F-actin microfilaments by Phalloidin or siRNAs-mediated decreased levels of CFL1 and DSTN enhanced osteoblast differentiation of hMSCs and heterotopic bone formation in vivo, while disruption of actin cytoskeleton by CytoD or inhibition of LIMK1 activity decreased osteoblast differentiation. In the current study, we observed opposite molecular changes associated with adipocyte differentiation i.e. enhancing actin F-filament stabilization inhibited adipocyte differentiation and decreased actin assembly enhanced adipocyte differentiation. These studies suggest an inverse relationship between the actin assembly state and the osteoblastic versus adipocytic differentiation potential of hMSC. A recent study reported that reduction of actin depolymerisation by CytoD treatment enhances commitment to both osteoblastic and adipocytic lineage based on increased expression of lineage specific markers and that a single intratibial injection of CytoD enhances bone formation and bone marrow adipocyte formation (Sen et al., 2015, 2017). This discrepancy may be

caused by differences in cellular composition of MSC cultures and/or culture conditions (short term versus long term treatment). Interestingly, our measurements of G/F actin during lineage differentiation in hMSCs showed similar reduction in actin polymerization (with increased G-actin in cells) during the first 3 days of osteoblast and adipocyte differentiation suggesting that initial differentiation phase of hMSCs and prior to specific lineage commitment associated with decreased actin assembly which is consistent with the published work of Sen et al. (2017). These authors reported that short-term treatment with CytoD increased the availability of G-actin and enhanced G-actin translocation to the nucleus that increased gene expression of both osteoblastic and adipogenic differentiation (Sen et al., 2017). However, different changes were observed later during the differentiation with formation of enriched cytoplasmic F-actin (Chen et al., 2015) and branching of nuclear actin that are associated with enhanced osteoblast differentiation. In addition, our findings of the presence of an inverse actin microfilaments dynamics during osteoblast versus adipocyte differentiation is more consistent with the notion of hMSCs lineage allocation to osteoblastic or adipocytic cells occur at the level of stem cells early during differentiation and associated with significant cytoskeletal changes (McBeath et al., 2004).

Changes in actin microfilaments are mediated by changes in extracellular matrix, mechanical stimulation or growth factors intracellular signaling. We observed significant changes in a number of intracellular signaling pathways associated with changes in actin dynamics during hMSCs differentiation. During adipocyte differentiation, decreased in actin assembly was associated with significant changes in Smad2 and ERK MAPK activation. Smad2 has been reported as a transcriptional modulator that represses CEBP activation which is an adipocyte master transcriptional factor. Consistent with this notion, we observed inhibition of Smad2 activation by CytoD and the enhancement of Smad2 activation by Phalloidin, suggested Smad2 signaling might be involved in the regulation of adipogenic differentiation of actin assembly. While SB431546, a TGF β inhibitor that inhibits TGF/Smad2 activation in cells, did not rescue the inhibition of adipogenic differentiation by Phalloidin, either the activation of phos-Smad2 activation by Phalloidin and adipogenic induction (data not shown), suggested the Smad2

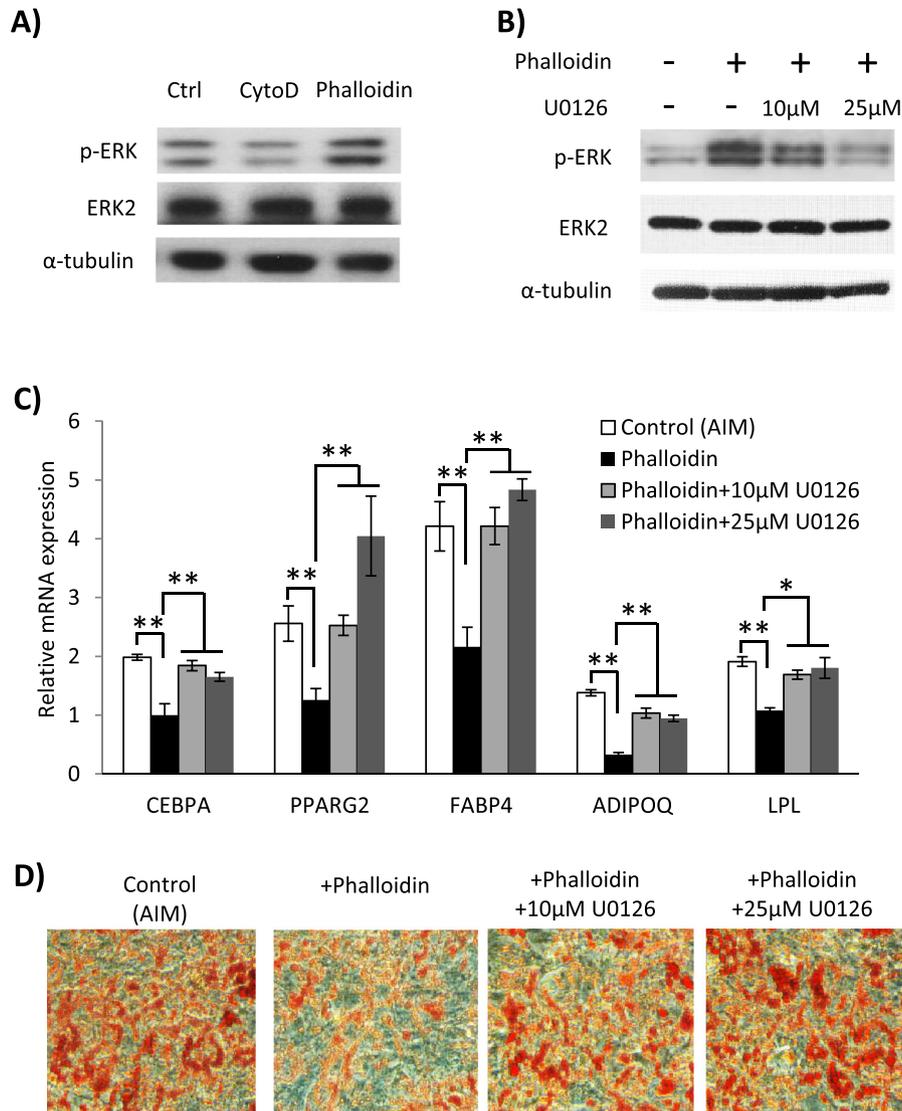


Fig. 5. Actin dynamics affects adipogenesis through ERK and Smad2. A. hMSCs was treated with DMSO as control, 0.5 μ M Cytochalasin D (CytoD) or 3 μ M Phalloidin and then underwent adipocyte differentiation for 10 days. The expression of total and phosphor-Smad2 and ERK were detected by western blot. α -tubulin was used as loading control. B–D. hMSCs was treated with adipogenic induction medium alone or in the presence of 3 μ M Phalloidin without or with either 10 or 25 μ M ERK inhibitor U0126. The total and phosphor-ERK were detected by western blot. α -tubulin was used as loading control (B). The expressions of adipogenic markers including CEBPA, ADIPOQ, LPL, PPARG and FABP4 were determined by qRT-PCR and normalized against B2M. Data was presented as mean \pm S.D., $n = 3$ (C). The accumulation of lipid droplets in mature adipocytes was visualized by Oil-red O staining (D). Representative data was shown from three independent experiments. * $P < .05$, ** $P < .01$.

activation by Phalloidin (actin polymerization) during adipogenic induction might not related with TGF β -TGFR pathways. Actin cytoskeleton has been reported to affect ERK activation in cell type dependent manner (Ailenberg and Silverman, 2003). In MSC, Muller et al. reported that CytoD enhances adipocyte differentiation in presence of mechanical stimulation and that these effects were associated with Akt but not ERK activation, while another actin depolymerizing reagent latrunculin A (LatA) blocked both Akt and ERK activation (Muller et al., 2013). We did not detected clear changes at activation of AKT, p38 or JNK by CytoD or Phalloidin in hMSCs during adipogenic differentiation (data not shown). These results indicate there are multiple signaling pathways associated with actin cytoskeletal changes dependent on cell type and culture conditions.

Changes in actin microfilament assembly and disassembly are associated with several cellular functions including cell cycle, morphological maintenance, cell attachment and adhesion, and cell locomotion which are determining factors for cellular differentiation fate (McBeath et al., 2004). While changing cell shape of hMSCs influences differentiation

potential of the cells as demonstrated in a number of studies where MSC were cultured on substrates of different stiffness (Engler et al., 2006). This approach has been utilized in tissue engineering protocols with the aim of controlling differentiation of MSC through engineering physical cues on cell culture surfaces or through exposing the cells to mechanical stimulation (Kshitiz et al., 2012). Our current study and previous study (Chen et al., 2015) demonstrate that by targeting the molecular mechanisms responsible for actin microfilaments assembly, it is possible to direct the differentiation fate of MSC into osteoblast or adipocytes. Thus, targeting intracellular molecular machinery responsible for cytoskeletal homeostasis is a plausible approach for controlling differentiation fate of MSC and is relevant approach for regenerative medicine applications (Fig. 6).

Acknowledgment

This study is supported by a grant from the Novo Nordisk Foundation (R179-A15165, L.C.), ECTS Postdoctoral Fellowship 2012 (L.C.)

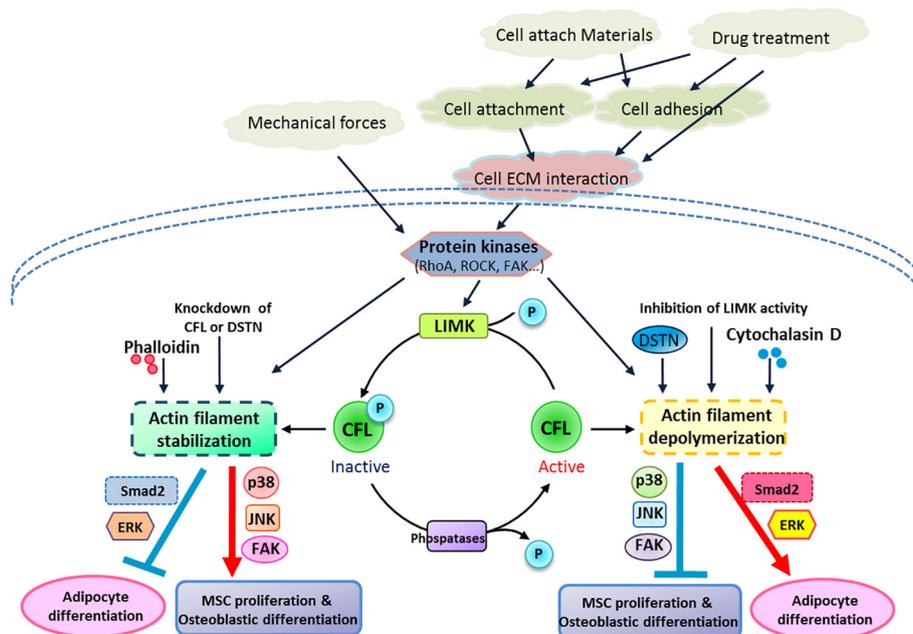


Fig. 6. The regulation of adipogenesis and osteogenesis by actin/CFL cytoskeleton dynamics. Extracellular chemical treatments or cell static physical cues, such as substrate attachment, cell adhesion, mechanical forces, can mediate those extracellular signaling through extracellular matrix (ECM) or binding with specific receptors, transfers the signaling to cell tension relative kinases (RhoA, Rock, FAK et al.), induce rearrangement of focal adhesions or activation of the downstream LIMK/CFL node, dynamically interconnecting with actin structure organization. Generally, actin depolymerisation factors (ADFs) binding to G-actin, interferes the actin filament polymerization, which inhibited the MSC proliferation and osteogenic differentiation, but enhanced the adipogenesis; while inactivation of CFL by phosphorylation at Ser3 site, or reduces the levels of ADFs expression, actin cytoskeleton shift to more stable polymerization, this induced cell proliferation and osteoblast differentiation, but inhibits the adipogenesis. The regulation signaling of actin cytoskeleton for osteogenesis is mediated by p38, JNK and FAK signaling pathways; while the regulation at adipogenesis is through Smad2 and ERK signaling pathway.

and Novo Nordisk Foundation (NNF150C0016284, M.K.). All authors have no conflicts of interest.

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