Biochemical and Biophysical Research Communications 464 (2015) 493–499

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Girdin/GIV is upregulated by cyclic tension, propagates mechanical signal transduction, and is required for the cellular proliferation and migration of MG-63 cells

Jiang-Tian Hu, Yan Li, Bing Yu, Guo-Jie Gao, Ting Zhou, Song Li*

Department of Orthodontics, School of Stomatology, Kunming Medical University, Kunming 650500, Yunnan, China

ARTICLE INFO

Article history:
Received 28 June 2015
Accepted 29 June 2015
Available online 7 July 2015

Keywords:
Cell migration assays
Cell proliferation
Cellular mechanotransduction
Human Girdin protein
Osteoblasts

ABSTRACT

To explore how Girdin/GIV is regulated by cyclic tension and propagates downstream signals to affect cell proliferation and migration. Human osteoblast-like MG-63 cells were exposed to cyclic tension force at 4000 μstrain and 0.5 Hz for 6 h, produced by a four-point bending system. Cyclic tension force upregulated Girdin and Akt expression and phosphorylation in cultured MG-63 cells. Girdin and Akt each promoted the phosphorylation of the other under stimulated tension. *In vitro* MTT and transwell assays showed that Girdin and Akt are required for cell proliferation and migration during cellular quiescence. Moreover, STAT3 was determined to be essential for Girdin expression under stimulated tension force in the physiological condition, as well as for osteoblast proliferation and migration during quiescence. These findings suggest that the STAT3/Girdin/Akt pathway activates in osteoblasts in response to mechanical stimulation and may play a significant role in triggering osteoblast proliferation and migration during orthodontic treatment.

1. Introduction

When loading a mechanical force onto teeth during orthodontic treatment, the alveolar bone is continuously remodeled. Osteoclasts resorb old bone on the side of pressure and osteoblasts form new bone on the side of tension. The current evidence suggests that during the process of force-induced bone remodeling, the cytoskeleton (comprised of actin filaments, microtubules, and intermediate filaments) can undergo complex deformation and biochemical change upon mechanical stimulation and convert mechanical signals into chemical signals. These signals subsequently regulate cell differentiation, proliferation, migration, and other cell phenotypes [1,2]. A host of molecules have been found to be involved in the signal transduction mechanism, like phosphatidylinositol 3-kinase (PI3K), Jun amino-terminal kinases [3], and cytoskeleton-related proteins, such as integrin-β and focal adhesion kinase [4]. Although significant progress has been made in recent years, our knowledge of mechanical signal transduction network remains far from complete.

Girders of actin filaments (Girdin) [5], also known as Gα-interacting vesicle-associated protein (GIV) [6], Akt-phoshorylation enhancer (APE) [7], or Hook-related protein 1 (HkRP1) [8], was first reported by four laboratories in 2005. *Girdin* is located in human chromosome 2p16.1 and encodes a large, 1870-amino acid protein with a predicted molecular mass of ~220–250 kDa [9]. Girdin is comprised of an α-helical coiled-coil domain (~1122 amino acids) in its middle conformation and a flanked N-terminal domain (~253 amino acids), as well as a C-terminal domain [9] (~495 amino acids). Girdin can crosslink with actin [5], linking actin filaments via the C-terminal half of its CT domain. In cortical cytoplasm, Girdin simultaneously associates with the plasma membrane via the N-terminal half of its CT domain [5]. These properties make Girdin an active participant in actin cytoskeletal remodeling during cell mobility. In addition, Girdin can activate Akt through two PI3K-dependent mechanisms [7,10]. Previous studies indicated that Girdin regulates diverse cell biological processes. It promotes transendothelial permeability [11], postnatal development of the dentate gyrus [12],

* Corresponding author. No. 1168 Chunrongxi Road, Chenggong District, Kunming City, Yunnan Province 650500, China.
E-mail address: song_li59@126.com (S. Li).

http://dx.doi.org/10.1016/j.bbrc.2015.06.165
0006-291X/

epithelial wound healing [13], macrophage chemotaxis [14], autophagy [15], tumor angiogenesis [16], cell proliferation [7], cell migration [5,17] and cancer invasion and metastasis [18].

Because of the close relationship between Girdin and the actin cytoskeleton (an essential mediator in mechanical signal transduction [1,2]), and the fact that Girdin can directly and indirectly activate the PI3K/Akt pathway (which has been shown to be activated in mechanical signal transduction [7,10]), here we hypothesize a new cell biological process for Girdin. We propose that Girdin is an active participant of mechanical signal transduction in the cell.

In this study, we examined the expression and interaction of Girdin and Akt in osteoblast-like cells under cyclic tensile stimulation. We then investigated the effects of Girdin and Akt proteins on osteoblast-like cell proliferation and migration by observing the behavior of the *Girdin* or *Akt*-depleted cells in MTT proliferation assays and transwell migration assays. Recent studies have identified that Girdin is a direct target of transcription factor signal transducer and activator of transcription-3 (STAT3) [13], so we also tested the expression of STAT3 under cyclic tensile stimulation and examined the effects of *STAT3* deletion on the stretch-induced expression and activation of Girdin.

2. Materials and methods

2.1. Antibodies and reagents

Anti-Girdin (1:2000) and anti-phosphor-Ser-1417 Girdin (1:2000) were provided by Abcam, Inc. (Cambridge, MA, USA). Anti-Akt2 (1:2000), anti-phosphor-Ser-474 Akt2 (1:2000), anti-STAT3 (1:500), anti-phosphor-Tyr-705 STAT3 (1:500), and anti-GAPDH (1:3000) were from Signalway Antibody (Pearland, TX, USA). Horseradish peroxidase-conjugated secondary antibody (1:10,000) were provided by Santa Cruz Biotechnology Inc. (Waltham, MA, USA). Sigma (St. Louis, MO, USA) supplied α-MEM, and polybrene. Fetal bovine serum (FBS) and Trizol reagent were from Gibco-BRL Corp. (Grand Island, NY, USA).

2.2. Cell culture

The human osteoblast-like MG-63 cell line was purchased from ATCC (ATCC number: CRL1427, Rockville, MD, USA). MG-63 cells were cultured in α-MEM supplemented with 10% FBS, 100U/mL penicillin, 100 μg/mL streptomycin, and 50 μg/mL ascorbic acid and were maintained in a humidified 37 °C incubator under 5% CO_2. The culture medium was changed once every three days throughout the experiment. After reaching 80% confluence, cells were detached and seeded into the force-loading plate at a density of 1×10^5 cells/cm^2. The force-loading plates were made as described previously [19].

2.3. RNA interference

The siRNA-mediated knockdown of Girdin and Akt was performed using previously described methods [5]. Briefly, the targeted sequences that effectively mediated the silencing of the expression of Girdin are as follows (only sense sequences are shown): 5′-AACCAGGTCATGCTCCAAATT-3′ (nucleotides 145–165, Girdin siRNA [A]) and 5′-AAGAAGGCTTAGGCAGGAATT-3′ (nucleotides 780–800, Girdin siRNA [B]). The 21-nucleotide synthetic duplexes were prepared by Qiagen. The siRNAs specific to human Akt2 and STAT3 were purchased from Qiagen. MG-63 cells were transfected with either 20 nM of three siRNAs or a 21-nucleotide irrelevant RNA (Qiagen) as a control for 48 h, using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. To achieve a good knockdown of the target, clones in which the expressions of Girdin, Akt2 and STAT3 were effectively suppressed (suppression > 50% of protein, as measured by Western blot) were selected and used for further study.

2.4. Cyclic tension stimulation

The Forcel Four-Point Bending Strength Device (West China College of Stomatology, Sichuan University, number of national patents of RP China: CN2534576 and CN1425905) was obtained commercially and used to apply mechanical tension to osteoblasts [19,20] (Fig. 1A and B). Herein, we plated MG-63 cells on force-loading plates and subjected them to cyclic uniaxial tension stimulation at 4000 μstrain and 0.5 Hz for 6 h by the four-point bending system. In the control groups, cells were cultured on similar plates and maintained in the same incubator without tensile loading. The operations were repeated in triplicate.

2.5. Western blot analysis

After cells were harvested, we extracted the protein and assayed it quantitatively using Bradford method. Protein extracts were separated on 10% SDS-polyacrylamide gels, followed by electrotransference onto polyvinylidene difluoride (PVDF) membranes (Roche Diagnostic, Germany). Membranes were probed with the primary antibody overnight at 4 °C. After incubation with a horseradish peroxidase-conjugated secondary antibody for 1 h at 37 °C, the immunoreactive bands were visualized by enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech). Phosphorylated Girdin (p-Girdin), Akt (p-Akt), and STAT3 (p-STAT3) were detected, and then, the PVDF membranes were stripped with eluent containing 99% β-mercaptoethanol, 10% SDS, and 1 M Tris–HCl (pH 6.7). A second hybridization was carried out to detect the levels of Girdin, Akt, STAT3 and GAPDH. Western blot images were quantified by using the Gel-Pro Analyzer software.

2.6. MTT cell proliferation assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay (Invitrogen) was performed according to the manufacturer's instructions. Therein, 2×10^3 to 6×10^3 cells were plated per well of a 96-well plate in full culture medium. To label the cells, MTT solution was added to the culture medium at a final concentration of 1.2 mM and incubation was

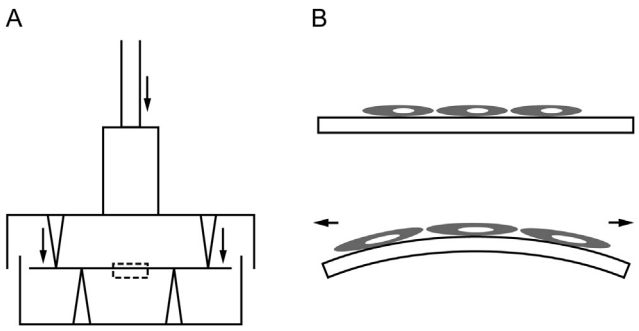

Fig. 1. Schematic representation of the Forcel Four-Point Bending Strength Device. A) Forcel Four-Point Bending Strength Device (West China College of Stomatology, Sichuan University, number of national patents of RP China: CN2534576 and CN1425905). This system can provide cyclic uniaxial tensile strain on adherent cell *in vitro*. Boxed area in panel A is enlarged in panel B. B) Tensile stress application. Therein, the force-loading plate and MG-63 cells upon it are deformed as load is applied to the plates.

continued for another 4 h. Dimethylsulfoxide was added and readings were taken on a plate reader at 490 nm. Readings were taken at specific time points (24, 48, and 72 h) after plating the cells.

2.7. Transwell migration assays

MG-63 cells (5×10^4 cells) were plated in the top chambers of Transwell® plates (BD Biosciences; pore diameter = 8 μm) in full culture medium minus EGF and were allowed to migrate downwards, towards medium supplemented with 20 ng/mL EGF, over a period of 24 h. At the end of the assay, cells in the top chamber were removed and the cells at the bottom of the filter were fixed with 100% ethanol for 10 min and stained with 0.1% crystal violet solution for 30 min. The dye was eluted using 33% acetic acid, and crystal violet absorbance was measured at 590 nm.

2.8. Statistical analysis

Experiments were repeated at least three times and results were expressed as mean ± standard deviation. Statistical significance between various conditions was evaluated with Student's *t*-test. $P < 0.05$ was considered significant.

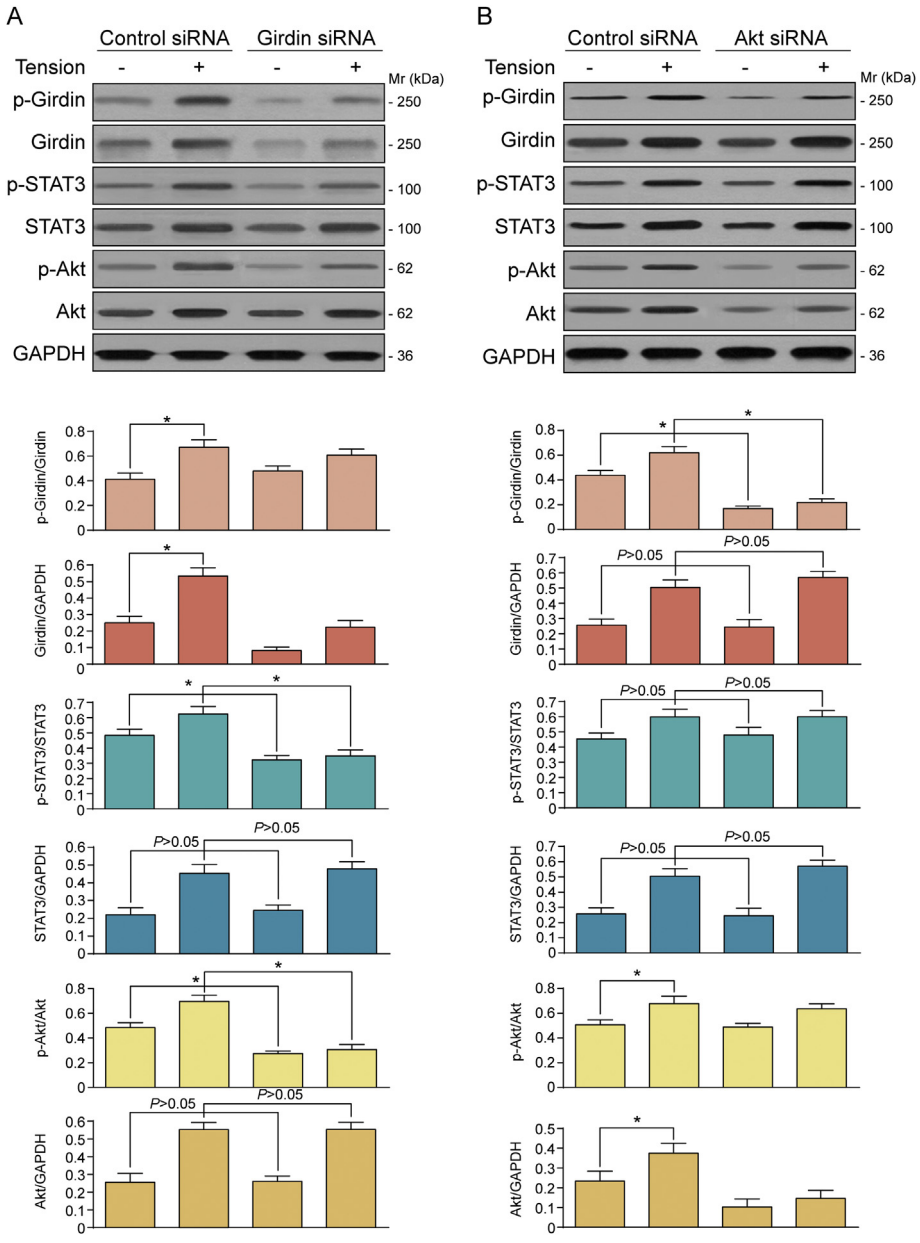

Fig. 2. Cyclic tension force upregulates Girdin and Akt expression and phosphorylation in human osteoblasts. A) MG-63 cells were transfected with Scr control siRNA (lanes 1, 2) and Girdin siRNA (lanes 3, 4) for 48 h. Then, cells were exposed to either tension force stimulation at 4000 μstrain and 0.5 Hz for 6 h by the four-point bending system (lanes 2, 4) or maintained in the same incubator without the tensile loading (lanes 1, 3). Finally, whole-cell lysates were analyzed for the indicated proteins by immunoblotting. B) MG-63 cells were transfected with Scr control siRNA (lanes 1, 2) and Akt siRNA (lanes 3, 4), then treated as above A. Whole-cell lysates were analyzed for the indicated proteins by immunoblotting. Changes in p-STAT3 and STAT3 levels caused by Girdin or Akt siRNA are described in Section 3.3. Assays were repeated at least in triplicate and gave similar results. Results were expressed as mean (column) ± standard deviation (error bar), $^*P < 0.05$.

3. Results

3.1. Cyclic tension force upregulates Girdin and Akt expression and phosphorylation in human osteoblasts

MG-63 cells were subjected to cyclic tension force at 4000 μstrain and 0.5 Hz. After 6 h, the protein expression levels of Girdin and Akt (relative to protein expression level of GAPDH) were increased by ~2.1- and 1.6-fold, respectively (Fig. 2A and B). Phosphorylation levels of Girdin and Akt (relative to the intensity level of the same protein) were increased ~1.6- and 1.5-fold, respectively, upon cyclic tension stimulation (Fig. 2A and B).

To determine the endogenous interaction between Girdin and Akt in osteoblasts under cyclic tension force, we performed RNA interference to suppress Girdin (Fig. 2A) or Akt (Fig. 2B) expression. Although the total Akt or Girdin level did not change significantly after Girdin or Akt knockdown, knockdown of Girdin attenuated the base-level and tension force-induced phosphorylation of Akt (Fig. 2A), and knockdown of Akt inhibited the base-level and tension force-induced phosphorylation of Girdin (Fig. 2B). Taken together, these data indicate that cyclic tension force is able to upregulate Girdin and Akt expression and phosphorylation in human osteoblasts, and that these two proteins can promote the phosphorylation of each other during quiescence and under conditions of tension stimulation.

3.2. Girdin and Akt are required for osteoblast proliferation and migration in quiescence

We next investigated the function of Girdin and Akt in quiescent osteoblasts by observing the behavior of *Girdin*- or *Akt*-depleted cells in MTT proliferation assays and transwell migration assays. We found that the *Girdin*- (Fig. 3A) or *Akt*-depleted (Fig. 3C) cells had significantly lower levels of proliferation compared to scrambled control siRNA-treated cells. In transwell migration assays, *Girdin*- (Fig. 3B) or *Akt*-depleted (Fig. 3D) cells also migrated less than the scrambled control siRNA-treated cells. Taken together, Girdin and Akt are essential for osteoblast proliferation and migration.

3.3. STAT3 is required for Girdin expression and osteoblast proliferation and migration

A recent study identified Girdin as a direct target of STAT3 [13]. Here, we investigated whether STAT3 is required for the upregulation of Girdin by cyclic tension-force stimulation. We found that tension-force stimulation upregulated the expression levels of STAT3 and Girdin, and increased the level of p-Girdin (Fig. 4A). Furthermore, knockdown of STAT3 reduced the base-level and force-induced expression levels of Girdin, but did not obviously influence the phosphorylation level of Girdin (Fig. 4A). In contrast, although knockdown of Girdin did not influence the expression of STAT3, the p-STAT3 level was significantly reduced under conditions of quiescence or tension stimulation (Fig. 2A). Knockdown of Akt did not obviously influence the expression or phosphorylation of STAT3 (Fig. 2B).

Next, we investigated whether STAT3 is also required for osteoblast proliferation and migration. Indeed, MTT proliferation assays showed that MG-63 cells transfected with siRNA against STAT3 have an impaired proliferation phenotype compared with cells transfected with control siRNA (Fig. 4B). In addition, in transwell migration assays, STAT3 siRNA-treated MG-63 cells migrated

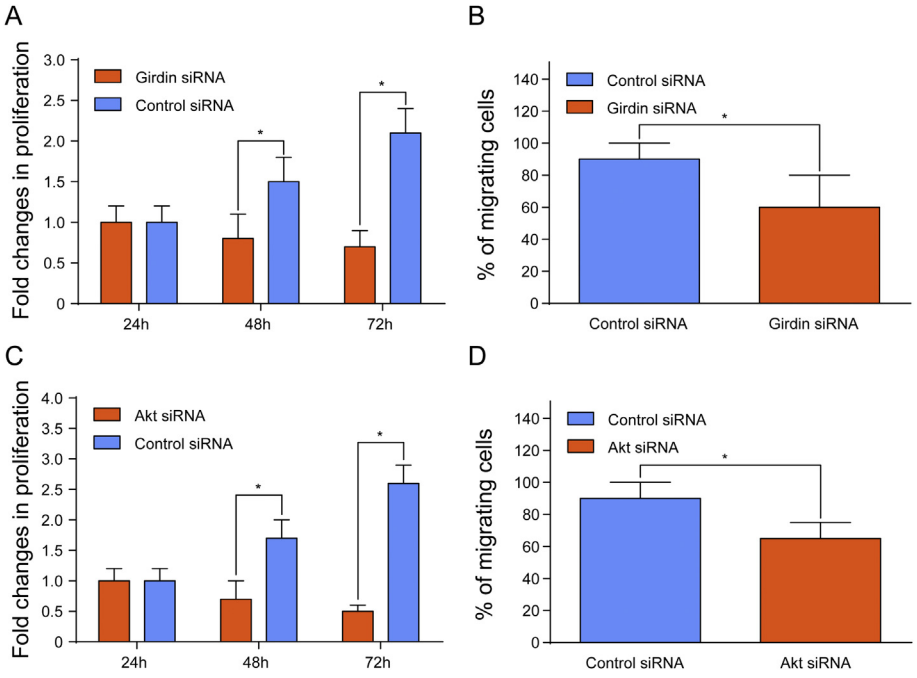

Fig. 3. Girdin and Akt are required for osteoblast proliferation and migration. A) Knockdown of Girdin attenuates osteoblast proliferation. We introduced Girdin siRNA and control siRNA into MG-63 cells, and then the total number of cells in the culture media at three observation points (24 h, 48 h, and 72 h after plating) was counted and expressed as fold changes in proliferation. B) Knockdown of Girdin impairs osteoblast migration. MG-63 cells treated with Girdin siRNA or control siRNA were plated in the top chamber of Transwell® migration plates in the absence of EGF and were allowed to migrate overnight towards medium supplemented with EGF in the lower chamber. The total number of cells in the lower chamber after 12 h was counted and expressed as the percent of cells analyzed. C) Knockdown of Akt attenuates osteoblast proliferation. To compare the proliferation phenotype of control and Akt siRNA cells, the MTT proliferation assays were performed as in A. D) Knockdown of Akt impairs osteoblast migration. To compare the migration phenotype of control and Akt siRNA cells, transwell migration assays were used as in B. The results are expressed as mean (column) ± standard deviation (error bar) of representative experiments out of at least $n = 3$. $^*P < 0.05$: Girdin or Akt siRNA vs. Control siRNA.

poorly compared with control siRNA-treated ones (Fig. 4C). Therefore, STAT3 is essential for Girdin expression in both quiescence and under tension force, and is required for osteoblast proliferation and migration.

4. Discussion

The main findings of the present study are: (1) that cyclic tensile force induces Girdin expression and phosphorylation in human osteoblast-like MG-63 cells (Fig. 2) and (2) depletion of Girdin by siRNA inhibits proliferation and migration of MG-63 cells (Fig. 3A and B). This is the first report investigating the change in and role of Girdin in MG-63 cells in response to cyclic tension force. A similar study reported that Gipie, a Girdin protein family member, is upregulated by mechanical stimulation and plays a protective role in the endoplasmic reticulum stress response in endothelial cells [21]. Others have reported that Girdin is phosphorylated on S1416 by Akt after the addition of growth factors (epithelial growth factor [5,22], insulin-like growth factor [18], vascular endothelial growth factor [16] and insulin [7,14,15,17]), whereas total Girdin levels do not change significantly under these conditions. In resting state cells, Takahashi et al. found that Girdin cross-links actin filaments, forms actin bundles diffusely in cytoplasm, and anchors these bundles to phosphatidylinositol 4-phosphate [PI(4)P] and phosphatidylinositol 3-phosphate [PI(3)P] in the plasma membrane at the cortical region of cells [5,9]. They also demonstrated that, in migrating cells induced by epithelial growth factor, the phosphorylation of Girdin at S1416 by Akt attenuates its affinity to PI(4)P and PI(3)P, but does not affect its affinity to actin filaments [5]. These studies were used to conclude that the changes in the affinity of Girdin to lipids cause it to detach from lipid-rich membranes (e.g., endoplasmic reticulum), move to the leading edge, and cross-link newly generated short actin filaments to facilitate lamellar protrusion and cell migration [9]. These previous results, combined with our findings in osteoblasts, lead us to propose that the upregulation and subsequent phosphorylation of Girdin in osteoblasts by cyclic tension force may directly contribute to actin cytoskeletal remodeling, which may subsequently trigger cell migration. Therefore, Girdin upregulation and phosphorylation are important events in the movement and manipulation of teeth by orthodontic practices.

Moreover, given the key role of osteoblasts in bone remodeling, their proliferation is considered an important marker of bone growth. Previous studies have reported that Girdin knockdown results in a reduction of DNA synthesis in HepG2 cells, with a corresponding decrease in Akt phosphorylation and activity [7]. These findings were supported by our conclusion that Girdin is required for MG-63 cell proliferation (Fig. 3A). The role of Girdin in regulating cell proliferation may be attributed to its ability to activate the PI3K/Akt pathway [10,17], which is well known to mediate cell

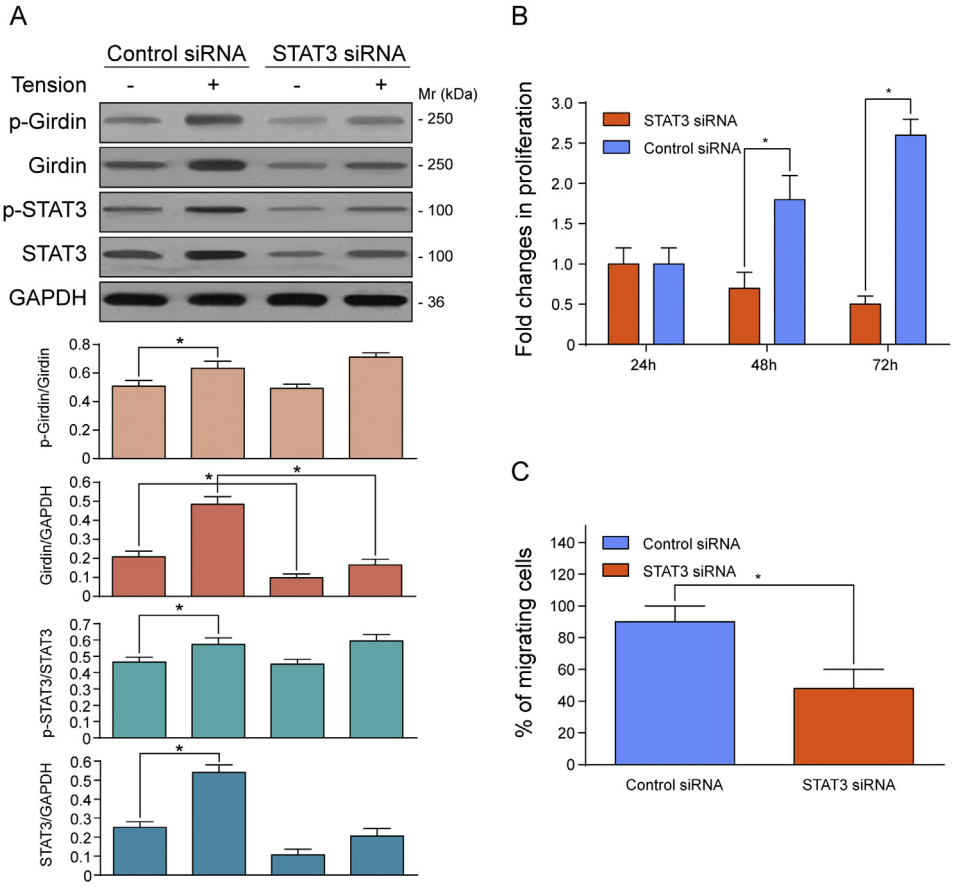

Fig. 4. STAT3 is required for Girdin expression and osteoblast proliferation and migration. A) MG-63 cells were transfected with Scr control siRNA (lanes 1, 2) and STAT3 siRNA (lanes 3, 4). Then the cells were exposed to either tension force stimulation at 4000 μstrain and 0.5 Hz for 6 h by the four-point bending system (lanes 2, 4) or maintained in the same incubator without the tensile loading (lanes 1, 3). Finally, whole-cell lysates were analyzed for the indicated proteins by immunoblotting. B) Knockdown of STAT3 attenuates osteoblast proliferation. To compare the proliferation phenotype of control and STAT3 siRNA cells, the MTT proliferation assays were performed as in Fig. 3A. C) Knockdown of Girdin impairs osteoblast migration. To compare the migration phenotype of control and STAT3 siRNA cells, transwell migration assays were used as in Fig. 3B. The results are expressed as mean (column) ± standard deviation (error bar) of representative experiments out of at least $n = 3$. $^*P < 0.05$.

proliferation [7,23,24]. Recent studies showed that this pathway is activated in MG-63 cells under tension force [3].

Until now, there have been two generally accepted PI3K-dependent mechanisms for how Girdin enhances Akt phosphorylation. First, Girdin interacts with and activates Gαi3 through its GEF motif [17]. The activation of Gαi3 mediates the dissociation of Gβγ from Girdin-Gαi3 complexes. Gβγ, in turn, directly activates the catalytic p110 subunit of PI3K, and then activates downstream Akt signaling [14,25]. Recent studies showed that Girdin can be phosphorylated by receptor tyrosine kinases at Y1764 and Y1798, and can directly interact with the regulatory p85 subunit of PI3K, thereby activating PI3K-dependent Akt signaling [10].

However, other authors found that the exogenously induced expression of both Girdin and Akt inhibits cell proliferation and induces apoptosis in COS-7 and HeLa cells [7], which was not observed in our research. This finding suggests that the role of Girdin in cell proliferation might be far more complex and, indeed, might be cell- and/or tissue-specific. Another study of the Girdin protein structure found that its C-terminal guanine nucleotide exchange factor (GEF) motif is required to enhance migration (motogenic) and suppress proliferation (mitogenic) pathways [22]. In the absence of GEF (e.g., in some cancers due to the alternative splicing of Girdin pre-mRNA), the remaining N-terminal Girdin residues are sufficient to propagate proliferation pathways [22].

We found that STAT3 is required for the expression of Girdin in MG-63 cells at the resting state or under cyclic tension force (Fig. 4), in line with Ghosh et al., who concluded that STAT3 is the transcription factor of Girdin [13]. In that study, overexpression (or knockdown) of STAT3 in HeLa cells increased (or decreased) the mRNA and protein expression levels of Girdin. STAT3 also immunoprecipitated with the *Girdin* promoter, most obviously at 6 h after induction of migration by a wound scratch. Interestingly, Ghosh et al. found that Girdin enhances STAT3 activation through its GEF motif, and forms a positive feedback loop that regulates Girdin's own expression [13]. These changes may explain, at least in part, the increased p-STAT3 level under tension-force stimulation. Of note, depletion of STAT3 did not completely abolish Girdin transcription, also observed by Ghosh et al. [13], suggesting that there may be other transcription factors and cofactor complexes that effectively and efficiently fine-tune Girdin expression.

We demonstrated that (1) cyclic tension force upregulates Girdin and Akt expression and phosphorylation in cultured human osteoblast-like MG-63 cells, and (2) these two proteins can promote the phosphorylation of each other after tension stimulation. (3) *In vitro* MTT and transwell assays showed that Girdin and Akt are required for cell proliferation and migration. (4) Moreover, for the first time, STAT3 was found to be essential for Girdin expression after application of the tension forces under physiological conditions, as well as required for osteoblast proliferation and migration. These findings suggest that the STAT3/Girdin/Akt pathway activates in osteoblasts in response to mechanical stimulation, and may play a significant role in triggering cell proliferation and migration during orthodontic treatment.

Source of funding

This work was supported by the National Natural Science Foundation of China (grant no. 81160137) and the Yunnan Applied Basic Research Project (grant no. 2011FB057) to J.-T. H. The funding source had no role in the manuscript preparation.

Conflict of interest

None.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.165.

References

[1] J.T. Hu, Cytoskeleton and its mechanical signal transduction, J. Kunming Med. Univ. 35 (2014) 1–3.
[2] M.C. Meikle, The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt, Eur. J. Orthod. 28 (2006) 221–240.
[3] L.W. Xiao, M. Yang, J. Dong, H. Xie, G.L. Sui, Y.L. He, J.X. Lei, E.Y. Liao, X. Yuan, Stretch-inducible expression of connective tissue growth factor (CTGF) in human osteoblasts-like cells is mediated by PI3K-JNK pathway, Cell. Physiol. Biochem. 28 (2011) 297–304.
[4] M. Yang, L.W. Xiao, E.Y. Liao, Q.J. Wang, B.B. Wang, J.X. Lei, The role of integrin-beta/FAK in cyclic mechanical stimulation in MG-63 cells, Int. J. Clin. Exp. Pathol. 7 (2014) 7451–7459.
[5] A. Enomoto, H. Murakami, N. Asai, N. Morone, T. Watanabe, K. Kawai, Y. Murakumo, J. Usukura, K. Kaibuchi, M. Takahashi, Akt/PKB regulates actin organization and cell motility via Girdin/APE, Dev. Cell 9 (2005) 389–402.
[6] H. Le-Niculescu, I. Niesman, T. Fischer, L. DeVries, M.G. Farquhar, Identification and characterization of GIV, a novel Galpha i/s-interacting protein found on COPI, endoplasmic reticulum-Golgi transport vesicles, J. Biol. Chem. 280 (2005) 22012–22020.
[7] M. Anai, N. Shojima, H. Katagiri, T. Ogihara, H. Sakoda, Y. Onishi, H. Ono, M. Fujishiro, Y. Fukushima, N. Horike, A. Viana, M. Kikuchi, N. Noguchi, S. Takahashi, K. Takata, Y. Oka, Y. Uchijima, H. Kurihara, T. Asano, A novel protein kinase B (PKB)/AKT-binding protein enhances PKB kinase activity and regulates DNA synthesis, J. Biol. Chem. 280 (2005) 18525–18535.
[8] F. Simpson, S. Martin, T.M. Evans, M. Kerr, D.E. James, R.G. Parton, R.D. Teasdale, C. Wicking, A novel hook-related protein family and the characterization of hook-related protein 1, Traffic 6 (2005) 442–458.
[9] A. Enomoto, J. Ping, M. Takahashi, Girdin, a novel actin-binding protein, and its family of proteins possess versatile functions in the Akt and Wnt signaling pathways, Ann. N. Y. Acad. Sci. 1086 (2006) 169–184.
[10] C. Lin, J. Ear, Y. Pavlova, Y. Mittal, I. Kufareva, M. Ghassemian, R. Abagyan, M. Garcia-Marcos, P. Ghosh, Tyrosine phosphorylation of the Galpha-interacting protein GIV promotes activation of phosphoinositide 3-kinase during cell migration, Sci. Signal 4 (2011) ra64.
[11] H. Ichimiya, K. Maeda, A. Enomoto, L. Weng, M. Takahashi, T. Murohara, Girdin/GIV regulates transendothelial permeability by controlling VE-cadherin trafficking through the small GTPase, R-Ras, Biochem. Biophys. Res. Commun. 461 (2015) 260–267.
[12] A. Enomoto, N. Asai, T. Namba, Y. Wang, T. Kato, M. Tanaka, H. Tatsumi, S. Taya, D. Tsuboi, K. Kuroda, N. Kaneko, K. Sawamoto, R. Miyamoto, M. Jijiwa, Y. Murakumo, M. Sokabe, T. Seki, K. Kaibuchi, M. Takahashi, Roles of disrupted-in-schizophrenia 1-interacting protein girdin in postnatal development of the dentate gyrus, Neuron 63 (2009) 774–787.
[13] Y. Dunkel, A. Ong, D. Notani, Y. Mittal, M. Lam, X. Mi, P. Ghosh, STAT3 protein up-regulates Galpha-interacting vesicle-associated protein (GIV)/Girdin expression, and GIV enhances STAT3 activation in a positive feedback loop during wound healing and tumor invasion/metastasis, J. Biol. Chem. 287 (2012) 41667–41683.
[14] P. Ghosh, M. Garcia-Marcos, S.J. Bornheimer, M.G. Farquhar, Activation of Galphai3 triggers cell migration via regulation of GIV, J. Cell. Biol. 182 (2008) 381–393.
[15] M. Garcia-Marcos, J. Ear, M.G. Farquhar, P. Ghosh, A GDI (AGS3) and a GEF (GIV) regulate autophagy by balancing G protein activity and growth factor signals, Mol. Biol. Cell. 22 (2011) 673–686.
[16] T. Kitamura, N. Asai, A. Enomoto, K. Maeda, T. Kato, M. Ishida, P. Jiang, T. Watanabe, J. Usukura, T. Kondo, F. Costantini, T. Murohara, M. Takahashi, Regulation of VEGF-mediated angiogenesis by the Akt/PKB substrate Girdin, Nat. Cell. Biol. 10 (2008) 329–337.
[17] M. Garcia-Marcos, P. Ghosh, M.G. Farquhar, GIV is a nonreceptor GEF for G alpha i with a unique motif that regulates Akt signaling, Proc. Natl. Acad. Sci. U S A 106 (2009) 3178–3183.
[18] P. Jiang, A. Enomoto, M. Jijiwa, T. Kato, T. Hasegawa, M. Ishida, T. Sato, N. Asai, Y. Murakumo, M. Takahashi, An actin-binding protein Girdin regulates the motility of breast cancer cells, Cancer Res. 68 (2008) 1310–1318.
[19] J. Liu, T. Liu, Y. Zheng, Z. Zhao, Y. Liu, H. Cheng, S. Luo, Y. Chen, Early responses of osteoblast-like cells to different mechanical signals through various signaling pathways, Biochem. Biophys. Res. Commun. 348 (2006) 1167–1173.
[20] X.H. Mi, Y. Wu, S.J. Luo, Z. Zhou, Y. Zheng, Osteoblast proliferation after different mechanical stimulation, J. Mod. Stomatol. 22 (2008) 609–611.
[21] E. Matsushita, N. Asai, A. Enomoto, Y. Kawamoto, T. Kato, S. Mii, K. Maeda, R. Shibata, S. Hattori, M. Hagikura, K. Takahashi, M. Sokabe, Y. Murakumo, T. Murohara, M. Takahashi, Protective role of Gipie, a Girdin family protein, in endoplasmic reticulum stress responses in endothelial cells, Mol. Biol. Cell. 22 (2011) 736–747.
[22] P. Ghosh, A.O. Beas, S.J. Bornheimer, M. Garcia-Marcos, E.P. Forry, C. Johannson, J. Ear, B.H. Jung, B. Cabrera, J.M. Carethers, M.G. Farquhar, A G

{alpha}i-GIV molecular complex binds epidermal growth factor receptor and determines whether cells migrate or proliferate, Mol. Biol. Cell. 21 (2010) 2338–2354.
[23] I. Vivanco, C.L. Sawyers, The phosphatidylinositol 3-Kinase AKT pathway in human cancer, Nat. Rev. Cancer 2 (2002) 489–501.
[24] J.R. Woodgett, Recent advances in the protein kinase B signaling pathway, Curr. Opin. Cell. Biol. 17 (2005) 150–157.
[25] P.J. Lilly, P.N. Devreotes, Chemoattractant and GTP gamma S-mediated stimulation of adenylyl cyclase in Dictyostelium requires translocation of CRAC to membranes, J. Cell. Biol. 129 (1995) 1659–1665.