Biochemical and Biophysical Research Communications 440 (2013) 677–682

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Acceleration of bone development and regeneration through the Wnt/β-catenin signaling pathway in mice heterozygously deficient for GSK-3β

Masaki Arioka [a,b], Fumi Takahashi-Yanaga [a,c,*], Masanori Sasaki [b], Tatsuya Yoshihara [a], Sachio Morimoto [a], Akihiko Takashima [d], Yoshihide Mori [b], Toshiyuki Sasaguri [a]

[a] Department of Clinical Pharmacology, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan
[b] Department of Oral and Maxillofacial Surgery, Faculty of Dental Science, Kyushu University, Fukuoka, Japan
[c] Global Medical Science Education Unit, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan
[d] Department of Aging Neurobiology, National Center for Geriatrics and Gerontology, Oobu, Japan

ARTICLE INFO

Article history:
Received 18 September 2013
Available online 4 October 2013

Keywords:
Bone regeneration
GSK-3β
Wnt/β-catenin signaling pathway

ABSTRACT

Glycogen synthase kinase (GSK)-3β plays an important role in osteoblastogenesis by regulating the Wnt/β-catenin signaling pathway. Therefore, we investigated whether GSK-3β deficiency affects bone development and regeneration using mice heterozygously deficient for GSK-3β (GSK-3β$^{+/-}$). The amounts of β-catenin, c-Myc, cyclin D1, and runt-related transcription factor-2 (Runx2) in the bone marrow cells of GSK-3β$^{+/-}$ mice were significantly increased compared with those of wild-type mice, indicating that Wnt/β-catenin signals were enhanced in GSK-3β$^{+/-}$ mice. Microcomputed tomography of the distal femoral metaphyses demonstrated that the volumes of both the cortical and trabecular bones were increased in GSK-3β$^{+/-}$ mice compared with those in wild-type mice. Subsequently, to investigate the effect of GSK-3β deficiency on bone regeneration, we established a partial bone defect in the femur and observed new bone at 14 days after surgery. The volume and mineral density of the new bone were significantly higher in GSK-3β$^{+/-}$ mice than those in wild-type mice. These results suggest that bone formation and regeneration *in vivo* are accelerated by inhibition of GSK-3β, probably through activation of the Wnt/β-catenin signaling pathway.

1. Introduction

Osteoblasts play a principal role in osseous tissue development and bone regeneration. They are derived from mesenchymal stem cells, and several cell signaling pathways are involved in regulation of osteoblastogenesis. Among them, the canonical Wnt pathway involving β-catenin (Wnt/β-catenin signaling pathway), which plays a number of key roles in embryonic development and maintenance of homeostasis in matured tissues [1], generates a major osteoblastogenic signal in osteoblast precursor cells [2,3].

Glycogen synthase kinase (GSK)-3 is a key regulator of the Wnt/β-catenin signaling pathway. GSK-3 controls the amount of β-catenin in the nucleus, which determines the intensity of the Wnt signal by phosphorylating β-catenin in the cytosol to trigger ubiquitin-dependent proteolysis of β-catenin [3]. GSK-3 inhibitors have been reported to stimulate osteoblastic differentiation of mesenchymal stem cells *in vitro* [2,4,5]. GSK-3 inhibitors also increase bone mass and mineral density *in vivo* [6–9]. Moreover, Kugiyama et al. reported that mice heterozygously deficient for GSK-3β (GSK-3β$^{+/-}$ mice) display accelerated bone formation during growth [10]. They also found that the activity of runt-related transcription factor-2 (Runx2), an essential transcription factor for osteoblastogenesis [11–13], is enhanced in GSK-3β$^{+/-}$ mice, because Runx2 is released from inhibitory phosphorylation by GSK-3β [10]. However, it remains unclear whether GSK-3β inhibition also accelerates bone regeneration after injury.

In the present study, we examined the effect of GSK-3β inhibition on bone development and regeneration. First, we compared the expression levels of Wnt/β-catenin target genes between wild-type and GSK-3β$^{+/-}$ mice to determine whether this pathway was activated in GSK-3β$^{+/-}$ mice. Second, we analyzed osseous tissue formation in GSK-3β$^{+/-}$ mice compared with that in wild-type mice to examine the effect of GSK-3β inhibition on bone development. Finally, we examined the effect of GSK-3β inhibition on bone regeneration using mice with an artificial bone defect. We compared the rate of regeneration and the quality of regenerated bone between wild-type and GSK-3β$^{+/-}$ mice.

* Corresponding author at: Department of Clinical Pharmacology, Faculty of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan. Fax: +81 92 642 6084.
E-mail address: yanaga@clipharm.med.kyushu-u.ac.jp (F. Takahashi-Yanaga).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.126

2. Materials and methods

2.1. Chemicals and antibodies

A monoclonal anti-histone H3 antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Monoclonal anti-β-catenin and anti-GSK-3β antibodies were purchased from BD Biosciences (San Jose, CA). A monoclonal anti-cyclin D1 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal anti-Runx2 and anti-c-Myc antibodies were purchased from Cell Signaling Technology (Danvers, MA). A monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Abcam (Cambridge, UK).

2.2. Generation of GSK-3$\beta^{+/-}$ mice

GSK-3$\beta^{+/-}$ mice were generated as described previously [14]. Heterozygous knockout of an GSK-3β allele was confirmed by PCR using mouse genomic DNA as described previously [14].

2.3. Purification of nuclear proteins

Nuclear proteins were isolated from bone marrow cells using NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL) according to manufacturer's instructions.

2.4. Western blotting

Bone marrow cells were collected from 8-week-old mouse femurs and lysed in Laemmli sample buffer. Protein samples (10 μg/lane) were separated by 12% SDS–polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membranes using a semi-dry transfer system (1 h at 12 V). Western blot analysis was performed as described previously [15]. Optical densitometric scans were performed using ImageJ software (National Institutes of Health, Bethesda, MD).

2.5. Microcomputed tomography (micro-CT)

Morphological analysis of mouse femurs was performed using a micro-CT system (Skyscan 1076 scanner; Skyscan, Konitich, Belgium). Scanning conditions were set to 48 kV, 201 μA, and 9 μm for one scan image. Three-dimensional (3D) reconstruction of images was performed with InstaRecon/NRecon software (Skyscan). Three regions were quantitatively analyzed in 8- (first and second) or 12-week-old mice (third): cortical bone regions from 2.0 to 2.5 mm above the growth plate at the distal metaphyses; trabecular bone regions from 0.1 to 1.1 mm above the growth plate at the distal metaphyses; injured cortical bone regions at 0.25 mm above and below the bone defect produced by a round bur of 1 mm in diameter (total analyzed width was 1.5 mm). We calculated the bone volume/total volume, bone surface/total volume, cortical thickness, cortical bone area/total cross-sectional area, trabecular number, trabecular thickness, trabecular separation, new bone volume/total bone volume, and bone mineral density. For each parameter, micro-CT-derived standard bone morphometry nomenclature, symbols, and units were used as described previously [16].

2.6. Establishment of bone injuries in mice

The legs of 12-week-old male wild-type and GSK-3$\beta^{+/-}$ C57BL/6 mice were shaved and sterilized with 70% ethanol, and then the mice were anesthetized to perform surgery. A 10 mm incision was made on the skin in front of the mid-femur. To expose the femoral surface, muscles were split to excise the periosteum. A defect was then established by drilling a hole into the mid-femur (5 mm above the knee joint) using a round dental bur of 1 mm in diameter. The surgical field was irrigated with saline, followed by suturing the incision line. The mice were sacrificed at 14 days after surgery and their femurs were removed for analysis.

2.7. Statistics

Results were expressed as the mean ± S.E.M. Differences between values were statistically analyzed by the Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

2.8. Ethics

The study protocol was approved by the Committee of Ethics on Animal Experiments of Kyushu University. Animal handling and procedures were carried out in compliance with the Guidelines for Animal Experiments, Kyushu University, and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

3. Results

3.1. Wnt/β-catenin signaling mediators are activated in GSK-3$\beta^{+/-}$ mice

Homozygous deficiency for GSK-3β (GSK-3$\beta^{-/-}$) yields an embryonic-lethal phenotype in mice, because of hepatocyte apoptosis or a cardiac patterning defect [17]. Therefore, we used GSK-3$\beta^{+/-}$ mice to investigate the role of GSK-3β in bone biology. Fig. 1A shows that the mice harbored both the normal and delete allele, and that the expression level of GSK-3β protein in these mice was about 50% of that in wild-type mice, which is consistent with a previous report [14].

The activity of the Wnt/β-catenin signaling pathway is dependent on the amount of β-catenin, which is controlled by GSK-3. As shown in Fig. 1B, the amounts of β-catenin (total, cytosolic, or nuclear) were all increased in GSK-3$\beta^{+/-}$ mice compared with those in wild-type mice, suggesting that GSK-3-induced degradation of β-catenin was attenuated in GSK-3$\beta^{+/-}$ mice. Next, we examined the expression levels of Wnt signal target genes cyclin D1, c-Myc, and Runx2 [1,18,19]. Their levels were all elevated in GSK-3$\beta^{+/-}$ mice compared with those in wild-type mice (Fig. 1C). These results strongly indicated that the Wnt/β-catenin signaling pathway was activated in GSK-3$\beta^{+/-}$ mice.

3.2. Enhancement of bone development in GSK-3$\beta^{+/-}$ mice

Next, we examined whether there is a difference in bone formation and development between wild-type and GSK-3$\beta^{+/-}$ mice. Distal metaphyses of the femoral bone of 8-week-old mice were scanned by a micro-CT system to evaluate the development of cortical and trabecular bones. Fig. 2A shows the coronal section images of the femur (left panel), transverse section images of the cortical bone (right upper panel), and 3D-reconstructed images of the trabecular bone (right lower panel). The volumes of both cortical and trabecular bones appeared to increase in GSK-3$\beta^{+/-}$ mice compared with those in wild-type mice. Morphological parameters that indicate facilitated bone formation were calculated from these images (Fig. 2B). Values for all of the parameters were elevated in GSK-3$\beta^{+/-}$ mice, suggesting that both cortical and trabecular bones underwent enhanced development in GSK-3$\beta^{+/-}$ mice compared with that in wild-type mice, which is consistent with a previous report by Kugiyama et al. [10].

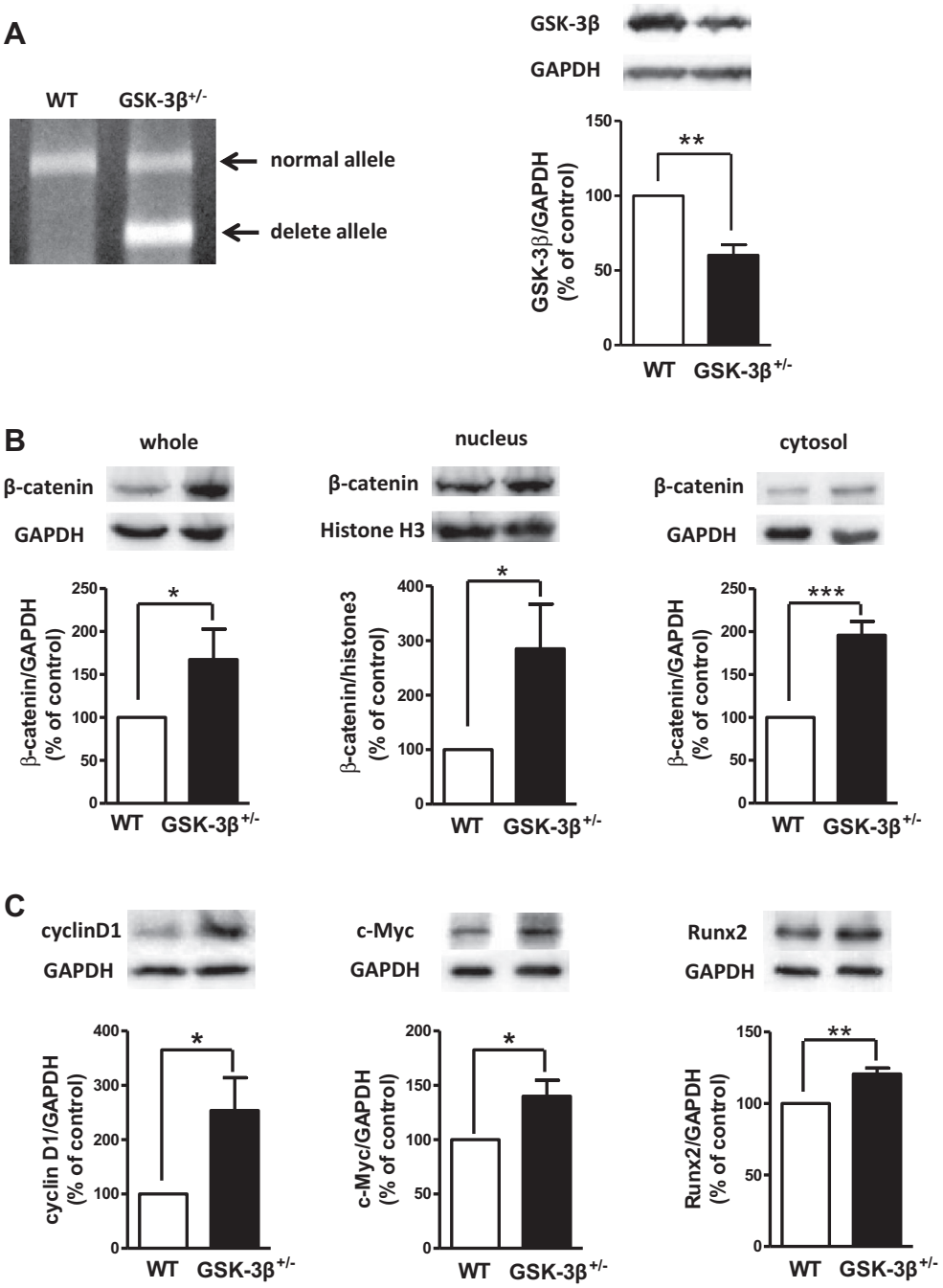

Fig. 1. Expression levels of Wnt signal-related proteins in bone marrow cells. (A) GSK-3β expression in GSK-3β$^{+/-}$ mice. Heterozygous knockout of a GSK-3β allele was confirmed by PCR (left panel). GSK-3β protein levels were determined by Western blot analysis using GAPDH as a loading control (right panel). Protein bands were quantified and shown as percentages of the control level in wild-type mice. Values are the mean ± S.E.M. (n = 3). (B) β-catenin levels in total, cytosolic, and nuclear protein fractions. β-catenin protein levels were determined by Western blot analysis using GAPDH or histone H3 as a loading control. Protein bands were quantified and shown as percentages of the control levels in wild-type mice. Values are the means ± S.E.M. (n = 4). (C) Levels of Wnt signal target proteins, cyclin D1, c-Myc, and Runx2, were determined by Western blot analysis using GAPDH as a loading control. Protein bands were quantified and shown as percentages of the control levels in wild-type mice. Values are the mean ± S.E.M. (n = 4). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. wild-type mice. WT, wild-type mice.

3.3. Acceleration of bone regeneration in GSK-3β$^{+/-}$ mice

To examine whether recovery from bone injury is accelerated in GSK-3β$^{+/-}$ mice, a round defect was established in the femurs of 12-week-old mice. Their femoral bones were scanned by micro-CT on the day of surgery (day 0) and after sacrifice (day 14) (Fig. 3A). The volume of regenerated bone in GSK-3β$^{+/-}$ mice was significantly larger than that in wild-type mice (Fig. 3B). Moreover, the density of regenerated bones in GSK-3β$^{+/-}$ mice was higher than that in wild-type mice (Fig 3C). Therefore, it appeared that GSK-3β deficiency accelerated regeneration of dense bones.

4. Discussion

In the present study, we found increases of both cortical and trabecular bone volumes in GSK-3β$^{+/-}$ mice compared with those in wild-type mice. Furthermore, we demonstrated for the first time that bone regeneration of a partial bone defect was accelerated and

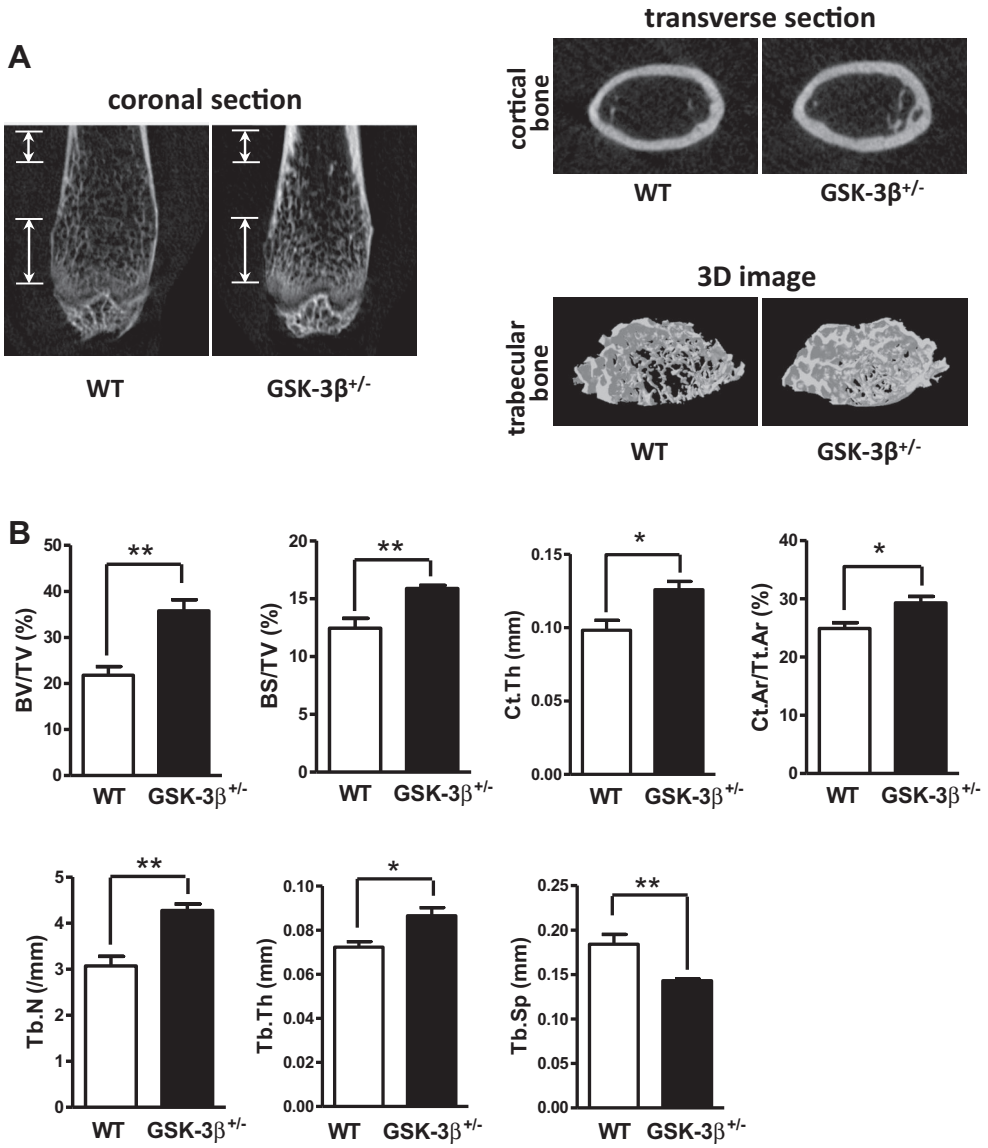

Fig. 2. Histomorphometric analyses of micro-CT images of the femoral bones. (A) Left panel shows coronal sections of the distal femoral metaphyses. Upper arrows indicate the width of the regions analyzed to calculate cortical bone parameters (from 2.0 to 2.5 mm above the growth plate in the distal metaphysis). Lower arrows indicate the width of the regions analyzed to calculate trabecular bone parameters (from 0.1 to 1.1 mm above the growth plate in the distal metaphysis). Upper right panel shows transverse sections of the cortical bone, and lower right panel shows 3D-reconstructed images of the trabecular bone. (B) Comparative analyses of histomorphometric parameters between wild-type and GSK-3$\beta^{+/-}$ mice. BV/TV, bone volume/total volume; BS/TV, bone surface/total volume; Ct.Th, cortical thickness; Ct.Ar/Tt.Ar, cortical bone area/total cross-sectional area; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation. Values are the means ± S.E.M. ($n = 7$). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. wild-type mice. WT, wild-type mice.

the mineral density of the regenerated bone was elevated in GSK-3$\beta^{+/-}$ mice compared with that in wild-type mice. Our results suggest that bone repair can be accelerated by inhibition of GSK-3β *in vivo*.

The Wnt/β-catenin signaling pathway plays a central role in bone formation [20–22] through up-regulation of Runx2 [19]. GSK-3β negatively regulates this pathway by facilitating β-catenin degradation [3,23]. Therefore, the mechanism for acceleration of bone formation and regeneration in GSK-3$\beta^{+/-}$ mice appears to be activation of the Wnt/β-catenin signaling pathway. Indeed, β-catenin was accumulated in nuclei, and the expression levels of Wnt target genes (cyclin D1, c-Myc, and Runx2) were elevated in GSK-3$\beta^{+/-}$ mice.

In contrast to our results, Kugiyama et al. reported that Runx2 is not increased in GSK-3$\beta^{+/-}$ mice [10]. This discrepancy may be caused by the difference in cell types subjected to Western blot analysis. Kugiyama et al. used calvarial osteoblasts, whereas we employed bone marrow cells that include osteoblast precursor cells. Because mature osteoblasts already express high levels of Runx2 [11], it may be difficult to detect a difference in Runx2 expression levels between GSK-3$\beta^{+/-}$ and wild-type mice.

Our results indicating that recovery of bone injury was accelerated in GSK-3β-deficient mice suggest that pharmacological inhibition of GSK-3β may facilitate healing of bone injuries. Some pharmacological agents that inhibit GSK-3β are available for basic research and clinical practice, e.g. AR28, 603281-31-8, and lithium carbonate. In fact, there are several reports of the effects of these inhibitors on osseous tissue. Systemic administration of AR28 and 603281-31-8 increases bone volume in mice [5,6]. Moreover, bone density is elevated in bipolar disorder patients treated with lithium carbonate for at least 1 year [9].

However, existing GSK-3β inhibitors are non-selective and also inhibit GSK-3α, another isozyme of GSK-3. The overall homology of GSK-3α (51 kDa) and GSK-3β (47 kDa) is approximately 85%, and

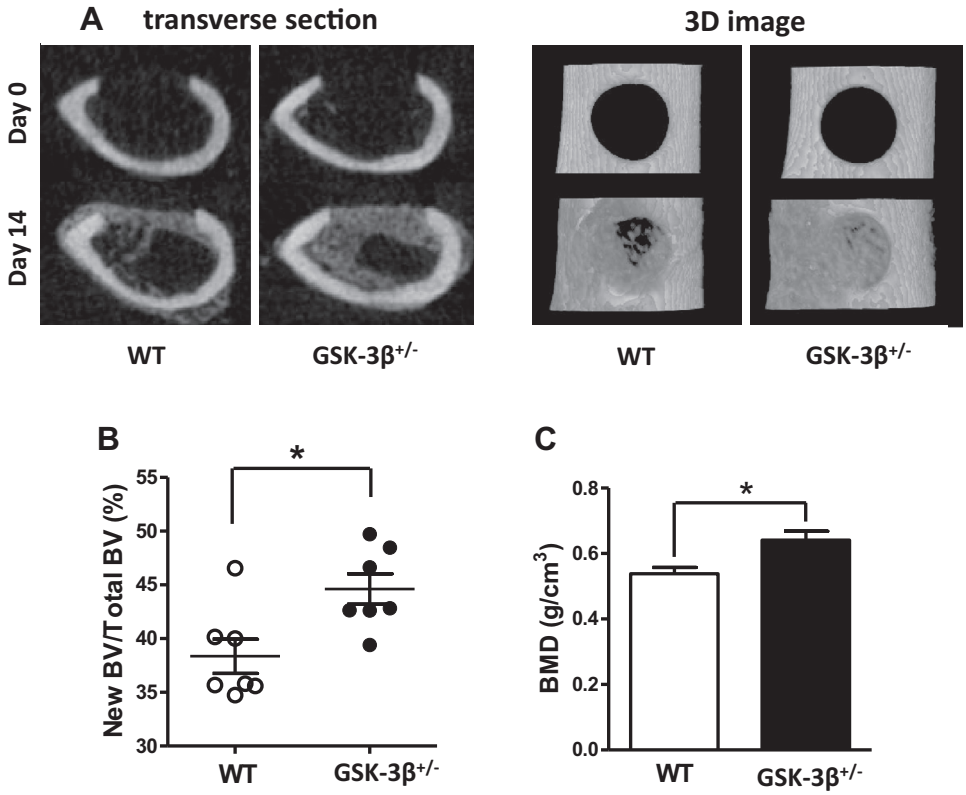

Fig. 3. Recovery of an artificial bone defect. (A) Representative micro-CT images at days 0 and 14 after injury. Left panel shows a horizontal image, and right panel shows a 3D- reconstructed image of the bone defect area. (B) Quantitative analysis of new bone volume. New BV/Total BV, new bone volume/total bone volume. (C) Quantitative analysis of new bone mineral density. BMD, bone mineral density. Values are the means ± S.E.M. ($n = 7$). $^*P < 0.05$ vs. wild-type mice. WT, wild-type mice.

the homology in the catalytic domain is approximately 98%. This very high homology in the catalytic domain causes difficulty to develop an inhibitor specific for one isozyme [24]. Functional differences between GSK-3α and GSK-3β in bone biology are unclear and there appears to be a very high level of functional redundancy for GSK-3 isozymes in terms of their participation in the Wnt/β-catenin signaling pathway [25]. In fact, similar to GSK-3$\beta^{+/-}$ mice [10], the bone volume in GSK-3α-deficient mice (GSK-3$\alpha^{-/-}$) is larger than that in wild-type mice [26], suggesting that their roles in bone formation may be similar. Therefore, non-selective GSK-3 inhibitors may be useful for the treatment of bone injury.

For clinical application of GSK-3 inhibitors, a critical consideration is adverse reactions caused by systemic administration [23]. Because GSK-3 is involved in a wide range of physiological functions, systemic administration of a GSK-3 inhibitor may induce serious adverse reactions unless a drug delivery system is used to specifically target the injured bone. In fact, oral administration of lithium carbonate can result in several adverse reactions, and its plasma concentration has to be monitored during treatment of bipolar disorder [27]. However, bone injury is a local disorder that does not require systemic administration of a medicine. Therefore, we might be able to devise a new therapeutic modality for bone injury by developing a topical application procedure for GSK-3 inhibitors.

In conclusion, our study suggests that recovery of bone injuries may be facilitated by suppression of GSK-3 activity, and that a novel pharmacotherapy using GSK-3 inhibitors might be developed for injured bone.

Acknowledgment

This work was supported by KAKENHI from the Ministry of Education, Culture, Sports, Science and Technology of Japan to F.T.Y. (25460334) and M.S. (25463090).

References

[1] C.Y. Logan, R. Nusse, The wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.
[2] G. Bain, T. Muller, X. Wang, J. Papkoff, Activated beta-catenin induces osteoblast differentiation of C3H10T1/2 cells and participates in BMP2 mediated signal transduction, Biochem. Biophys. Res. Commun. 301 (2003) 84–91.
[3] J.J. Westendorf, R.A. Kahler, T.M. Schroeder, Wnt signaling in osteoblasts and bone diseases, Gene 341 (2004) 19–39.
[4] G. Rawadi, B. Vayssiere, F. Dunn, R. Baron, S. Roman-Roman, BMP-2 controls alkaline phosphatase expression and osteoblast mineralization by a wnt autocrine loop, J. Bone Miner. Res. 18 (2003) 1842–1853.
[5] A. Gambardella, C.K. Nagaraju, P.J. O'Shea, S.T. Mohanty, L. Kottam, J. Pilling, M. Sullivan, M. Djerbi, W. Koopmann, P.I. Croucher, I. Bellantuono, Glycogen synthase kinase-3alpha/beta inhibition promotes *in vivo* amplification of endogenous mesenchymal progenitors with osteogenic and adipogenic potential and their differentiation to the osteogenic lineage, J. Bone Miner. Res. 26 (2011) 811–821.
[6] N.H. Kulkarni, J.E. Onyia, Q. Zeng, X. Tian, M. Liu, D.L. Halladay, C.A. Frolik, T. Engler, T. Wei, A. Kriauciunas, T.J. Martin, M. Sato, H.U. Bryant, Y.L. Ma, Orally bioavailable GSK-3alpha/beta dual inhibitor increases markers of cellular differentiation *in vitro* and bone mass *in vivo*, J. Bone Miner. Res. 21 (2006) 910–920.
[7] R. Nakanishi, H. Akiyama, H. Kimura, B. Otsuki, M. Shimizu, T. Tsuboyama, T. Nakamura, Osteoblast-targeted expression of Sfrp4 in mice results in low bone mass, J. Bone Miner. Res. 23 (2008) 271–277.
[8] P. Clement-Lacroix, M. Ai, F. Morvan, S. Roman-Roman, B. Vayssiere, C. Belleville, K. Estrera, M.L. Warman, R. Baron, G. Rawadi, Lrp5-independent activation of wnt signaling by lithium chloride increases bone formation and bone mass in mice, Proc. Natl. Acad. Sci. USA 102 (2005) 17406–17411.
[9] A. Zamani, G.R. Omrani, M.M. Nasab, Lithium's effect on bone mineral density, Bone 44 (2009) 331–334.
[10] F. Kugimiya, H. Kawaguchi, S. Ohba, N. Kawamura, M. Hirata, H. Chikuda, Y. Azuma, J.R. Woodgett, K. Nakamura, U.I. Chung, GSK-3beta controls osteogenesis through regulating Runx2 activity, PLoS One 2 (2007) e837.
[11] P. Ducy, R. Zhang, V. Geoffroy, A.L. Ridall, G. Karsenty, Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation, Cell 89 (1997) 747–754.
[12] T. Komori, H. Yagi, S. Nomura, A. Yamaguchi, K. Sasaki, K. Deguchi, Y. Shimizu, R.T. Bronson, Y.H. Gao, M. Inada, M. Sato, R. Okamoto, Y. Kitamura, S. Yoshiki, T. Kishimoto, Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts, Cell 89 (1997) 755–764.

[13] F. Otto, A.P. Thornell, T. Crompton, A. Denzel, K.C. Gilmour, I.R. Rosewell, G.W. Stamp, R.S. Beddington, S. Mundlos, B.R. Olsen, P.B. Selby, M.J. Owen, Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development, Cell 89 (1997) 765–771.
[14] T. Kimura, S. Yamashita, S. Nakao, J.M. Park, M. Murayama, T. Mizoroki, Y. Yoshiike, N. Sahara, A. Takashima, GSK-3beta is required for memory reconsolidation in adult brain, PLoS One 3 (2008) e3540.
[15] F. Takahashi-Yanaga, Y. Taba, Y. Miwa, Y. Kubohara, Y. Watanabe, M. Hirata, S. Morimoto, T. Sasaguri, Dictyostelium differentiation-inducing factor-3 activates glycogen synthase kinase-3beta and degrades cyclin D1 in mammalian cells, J. Biol. Chem. 278 (2003) 9663–9670.
[16] M.L. Bouxsein, S.K. Boyd, B.A. Christiansen, R.E. Guldberg, K.J. Jepsen, R. Muller, Guidelines for assessment of bone microstructure in rodents using micro-computed tomography, J. Bone Miner. Res. 25 (2010) 1468–1486.
[17] O. Kaidanovich-Beilin, J.R. Woodgett, GSK-3: functional insights from cell biology and animal models, Front. Mol. Neurosci. 4 (2011) 40.
[18] A. Kikuchi, H. Yamamoto, S. Kishida, Multiplicity of the interactions of wnt proteins and their receptors, Cell. Signal. 19 (2007) 659–671.
[19] T. Gaur, C.J. Lengner, H. Hovhannisyan, R.A. Bhat, P.V. Bodine, B.S. Komm, A. Javed, A.J. van Wijnen, J.L. Stein, G.S. Stein, J.B. Lian, Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression, J. Biol. Chem. 280 (2005) 33132–33140.
[20] Y. Gong, R.B. Slee, N. Fukai, G. Rawadi, S. Roman-Roman, A.M. Reginato, H. Wang, T. Cundy, F.H. Glorieux, D. Lev, M. Zacharin, K. Oexle, J. Marcelino, W. Suwairi, S. Heeger, G. Sabatakos, S. Apte, W.N. Adkins, J. Allgrove, M. Arslan-Kirchner, J.A. Batch, P. Beighton, G.C. Black, R.G. Boles, L.M. Boon, C. Borrone, H.G. Brunner, G.F. Carle, B. Dallapiccola, A. De Paepe, B. Floege, M.L. Halfhide, B. Hall, R.C. Hennekam, T. Hirose, A. Jans, H. Juppner, C.A. Kim, K. Keppler-Noreuil, A. Kohlschuetter, D. LaCombe, M. Lambert, E. Lemyre, T. Letteboer, L. Peltonen, R.S. Ramesar, M. Romanengo, H. Somer, E. Steichen-Gersdorf, B. Steinmann, B. Sullivan, A. Superti-Furga, W. Swoboda, M.J. van den Boogaard, W. Van Hul, M. Vikkula, M. Votruba, B. Zabel, T. Garcia, R. Baron, B.R. Olsen, M.L. Warman, Osteoporosis-Pseudoglioma Syndrome Collaborative Group. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development, Cell 107 (2001) 513–523.
[21] M.P. Whyte, W.H. Reinus, S. Mumm, High-bone-mass disease and LRP5, N. Engl. J. Med. 350 (2004) 2096–2099. author reply 2096–2099.
[22] M.L. Johnson, K. Harnish, R. Nusse, W. Van Hul, LRP5 and wnt signaling: a union made for bone, J. Bone Miner. Res. 19 (2004) 1749–1757.
[23] F. Takahashi-Yanaga, Activator or inhibitor? GSK-3 as a new drug target, Biochem. Pharmacol. 86 (2013) 191–199.
[24] H. Eldar-Finkelman, A. Martinez, GSK-3 inhibitors: preclinical and clinical focus on CNS, Front. Mol. Neurosci. 4 (2011) 32.
[25] B.W. Doble, S. Patel, G.A. Wood, L.K. Kockeritz, J.R. Woodgett, Functional redundancy of GSK-3alpha and GSK-3beta in Wnt/beta-catenin signaling shown by using an allelic series of embryonic stem cell lines, Dev. Cell 12 (2007) 957–971.
[26] J. Zhou, T.A. Freeman, F. Ahmad, X. Shang, E. Mangano, E. Gao, J. Farber, Y. Wang, X.L. Ma, J. Woodgett, R.J. Vagnozzi, H. Lal, T. Force, GSK-3alpha is a central regulator of age-related pathologies in mice, J. Clin. Invest. 123 (2013) 1821–1832.
[27] M. Schou, Lithium treatment at 52, J. Affect. Disord. 67 (2001) 21–32.