Cell Tissue Bank
DOI 10.1007/s10561-014-9449-6

ORIGINAL PAPER

Inactivation of glycogen synthase kinase-3β up-regulates β-catenin and promotes chondrogenesis

Junjie Zhou · Yan Chen · Chengfu Cao · Xianqi Chen · Wenwu Gao · Lei Zhang

Received: 25 November 2013 / Accepted: 14 April 2014

Abstract This study aimed to investigate whether inhibition of glycogen synthase kinase-3β (GSK-3β) could promote chondrocytes proliferation. The expression pattern of GSK-3β was firstly determined by immunohistochemistry (IHC) in normal mouse. Tibias were then isolated and cultured for 6 days. The tibias were treated with dimethylsulfoxide (control) or GSK-3 inhibitor SB415286 (SB86). Length of tibias was measured until 6 days after treatment. These bones were either stained with alcian blue/alizarin red or analyzed by IHC. In addition, GSK-3β and β-catenin were analyzed by Western blot. Finally, cartilage-specific GSK-3β deletion mice (KO) were generated. Efficiency of GSK-3β deletion was determined through Western blot and IHC. After treated by inhibitor SB86, the overall length of growth plate was not changed. However, growth of tibia in SB86 group was increased by 31 %, the length of resting and proliferating was increased 13 % ($P < 0.01$), whereas the length of hypertrophic was decreased by 57 % ($P < 0.01$). Besides, the mineralized length was found to be significant longer than the control group ($P < 0.05$). In KO mice, growth plate and calvaria tissue both exhibit significant reduction of GSK-3β ($P < 0.05$) whereas the lengths of tibias in KO were almost same compared with control mice. Finally, an increase amount of β-catenin protein was observed in SB86 ($P < 0.05$). In addition, significantly increased β-catenin was also found in the growth plate of KO mice ($P < 0.05$). Inhibition of GSK-3 could promote longitudinal growth of bone through increasing bone formation. Besides, the inactivation of GSK-3β could lead to enhancing β-catenin, therefore promote chondrocytes proliferation.

Keywords Glycogen synthase kinase-3β · β-catenin · Tibia · Chondrogenesis

J. Zhou (✉) · Y. Chen · C. Cao · X. Chen · W. Gao · L. Zhang
Orthopedic Surgery, PuTuo Hospital, Shanghai University of Traditional Chinese Medicine, No. 164, LanXi Road, Shanghai 200062, China
e-mail: junjiezhou33@hotmail.com

Introduction

In rheumatoid arthritis (RA) patients, the homeostasis of articular cartilage is always disrupted by chronic inflammation caused synovium and synovia infiltration, synovial tissue hyperplasia and monocytes activation (Bhukhai et al. 2012). The stable structure and function of cartilaginous tissue are provided by extracellular matrix which is produced and maintained by articular chondrocytes (Lewis et al. 2011; Toh et al. 2010). Articular chondrocytes are regulated by a variety of growth factors, especially Wnt family members. As reported, β-catenin as a number of Wnt family plays a critical role in multiple steps during chondrogenesis and chondrocyte maturation (Studer et al. 2012; Taipaleenmäki et al. 2011).

Wnt/β-catenin pathway is a highly conserved pathway, which could inhibits the degradation of β-

Published online: 24 April 2014

catenin and regulate transcription of a number of genes (Moon et al. 2002). As reported, β-catenin is essential in determining whether mesenchymal progenitors will become osteoblasts or chondrocytes (Hill et al. 2005; Day et al. 2005). Multiple genetic and pharmacological manipulations of Wnt signaling in mice have confirmed the central role of this pathway in regulating bone formation (Baron and Rawadi 2007). β-catenin are tightly regulated by Wnt mediated activity of "destruction complex", Once Wnt ligand binding to its receptors, the complex is disassociated and the kinase activity of GSK-3β is suppressed, then non-phosphorylated β-catenin is accumulated in cytoplasm and translocates into the nucleus. Finally Wnt target genes are activated, and lead to dysregulation of osteoblast/chondrocyte function (Primot et al. 2000). Glycogen synthases kinase-3β (GSK-3β) is involved in multiple physiological processes and could regulate various molecular pathways, such as Wnt, Hedgehog, NFAT, and insulin signaling (Wai and Kuo 2008). It was reported GSK-3β is closely related protein-serine kinases, which act as inhibitory components of Wnt signalling [04]. GSK-3β in a "destruction complex" together with Axin and APC could catalyze continuous proteasome-mediated degradation of phosphorylated β-catenin and lead to limited β-catenin expression (Pasco et al. 2010). Besides, GSK-3β was also shown to be critical in chondrocytes proliferation and ossification development (Naves et al. 2011). Therefore, chondrocytes may be influenced by GSK-3β through Wnt/β-catenin signaling.

In this study, the expression pattern of GSK-3β in normal growth plate was firstly determined. Tibias were cultured in vitro and the morphology was compared between GSK-3 inhibitor SB86 group and normal group. Besides, the expression of β-Catenin was also detected. Finally, cartilage-specific loss of GSK-3β mice were generated and the expression of GSK-3β was examined. We speculate that the inactivation of GSK-3β could lead to enhancing expression of β-catenin, and promote chondrocytes proliferation.

Materials and methods

Breeding and genotyping of mice

Homozygous Gsk3bfl/fl (Gsk3β alleles) mice (Xiao et al. 2010) were crossed with cartilage-specific (Col 2a1 promoter, expressing cre recombinase) mice. Gsk3b heterozygous mice (expressing Col 2a1 cre) were backcrossed with homozygous Gsk3b mice. The offsprings from these crosses were analyzed [15–16]. This breeding scheme produced knockout mice (KO) (Gsk3bfl/fl cre+) and control mice (Gsk3bfl/wt cre+). Mice were exposed in a 12-h light, 12-h dark cycle environment and fed with tap water and regular chow ad libitum. All procedures involving animals were approved by Shanghai University of TCM. PCR genotyping was performed using ear notch DNA by GSK-3β primers: 5′-GGGGCAACCTTAATTTCATT-3′ (forward) and 5′-TCTGGGCTATAGCTA TCTAGTAAC G-3′ (reverse), including 30 cycles of 55 s at 96 °C, 45 s at 56.5 °C, and 2 min 45 s at 68 °C. The transgenic of cre was detected by primers: 5′-CACACTGTGTAGT GCTTCGT-3′ (forward) and 5′-CCTCCAAACCATC-CAAG AT-3′ (reverse), including 40 cycles of 45 s at 95 °C, 30 s at 58 °C, and 1 min at 72 °C.

Culture of tibias

Tibias were isolated from embryonic mice (n = 5) and cultured for 6 days in serum-free medium containing α-MEM, ascorbic acid, β-glycerophosphate, BSA, glutamine, and penicillin–streptomycin without exogenous growth factors as described (Nogami et al. 2012). After dissection, tibias were incubated in medium overnight and then treated with dimethylsulfoxide (control, n = 9) or the 10 μM GSK-3 inhibitor SB415286 (SB86, n = 9; Boku et al. 2009). The media were changed every 48 h. Length of tibias was measured by callipers until 6 days after treatment.

Immunohistochemistry and staining

Cultured tibias were fixed in 4 % paraformaldehyde overnight and decalcified with 0.1 M EDTA/PBS at room temperature before paraffin embedding and sectioning at the medical institution of Shanghai University of TCM. Five-micrometer longitudinal sections of tibias were dewaxed in xylene followed by a graded series of ethanol washes (100 % twice, 95 % once, and 70 % once) (Park et al. 2012). For IHC, sections were incubated in 3 % H_2O_2 for 15 min at room temperature, followed by antigen retrieval by incubation in 10 mM sodium citrate at 95 °C for 30 min and 0.1 % Triton X-100 for 10 min, followed by blocking with 5 % goat serum in PBS. Sections

were incubated with primary antibody (GSK-3β, no. 9315/β-catenin, no. 9562. Sigma, Germany) overnight at 4 °C and washed (four times) in PBS, secondary antibody was applied according to manufacturers' recommendations. For detection, diaminobenzidine substrate was used and counterstained with methyl blue. Tissue staining was performed as described previously (Oseni et al. 2013). Cultured tibias isolated from embryos were dehydrated in 95 % ethanol for 24 h, followed by acetone for 24 h and stained with alcian blue/alizarin red. The stained tibias were stored in glycerol/ethanol (1:1). Images were taken with a Nikon SMZ1500 dissecting microscope with a Photometrics (Tucson, AZ, USA) Coolsnap camera using Image Master version 5.0.

Western blot analyses

Growth plate and calvarial tissues were dissected from (survival after postnatal) P0 mice of KO and control mice (n = 3) in cold Puck's solution A. Samples were flash frozen in RIPA buffer and stored at −20 °C overnight and then homogenized, sonicated, and centrifuged. Total protein content was determined, and 25–35 μg total protein (depending on protein yield) was loaded per lane in precast NuPAGE Novex Midi Tris–acetate gels and separated using the X-Cell Surelock Mini-cell (Invitrogen) system. Gels were blotted using X-Cell II Blot Module (Invitrogen) as the manufacturer's instructions. Blots were blocked in 5 % BSA Tris-buffered saline/Tween 20 solution for 1 h and then probed with primary antibody (GSK-3β/β-catenin) overnight at 4 °C. After washing (Tris-buffered saline/Tween 20), membranes were incubated with appropriate secondary antibody (hrp conjugated) for 1 h at room temperature, and the resultant signal was detected using the ECL detection system (Amersham, Piscataway, NJ, USA). Quantitative densitometry analysis was conducted using a ChemiImager 5500 system (Alpha Innotech, Miami, FL), subtracting background and normalizing to β-actin loading control signal. The Western blot was repeated for three times.

Statistical analysis

All data were collected from at least three independent cultured organ trial or pairs of littermates. Data are expressed as mean ± SE, and P values <0.05 were considered significant. For general measurements and comparisons between two groups of data, statistical significance was determined by unpaired t test to comparing control with treated (SB86) or control with KO littermates using GraphPad Prism version 3.00 for Windows. Western blot densitometry data were normalized to controls and compared by t test. Comparison of multiple treatments was done using one-way ANOVA (normal distribution analyzed) and a Tukey posttest.

Results

Expression of GSK-3β in the growth plate

To determine the expression pattern of GSK-3β in the growth plate in vivo, IHC was performed on wild-type P0 rat tibias. As a result, GSK-3β expressed at low levels in chondrocytes at the articular surface and can hardly detected in the resting and proliferating (R/P) chondrocytes. However, it highly expressed in prehypertrophic and hypertrophic (H) chondrocytes. In cellular level of hypertrophic chondrocytes, GSK-3β is found in cytoplasmic and especially in nuclear (Fig. 1).

Inhibition of GSK-3β affects ossification development in tibias

To determine the role of GSK-3β in endochondral bone growth, tibia organs were cultured for 6 days with the GSK-3β inhibitor. As show in Fig. 2, the overall length of the growth plate in treated tibia was not significantly changed. However, growth of tibia in SB86 group was increased by 31 % during 6 days treatment. The division between the R/P region was not distinct in treated tibias, so the R/P region was measured as a whole part. As a result, the length of R/P in the inhibitor treated tibia were 13 % longer than the controls, whereas the length of hypertrophic (H) was significantly decreased by 57 %. Because the inhibition of GSK-3 caused increased tibia growth but not the total growth plate length, we then examined the mineralized portion of the tibia. As a result, the mineralized length of the SB86 tibia was found to be significantly longer than the control group.

Chondrocyte-specific ablation of GSK-3β in vivo

To determine whether the results from cultured vitro tibia organs would represent actual conditions in vivo,

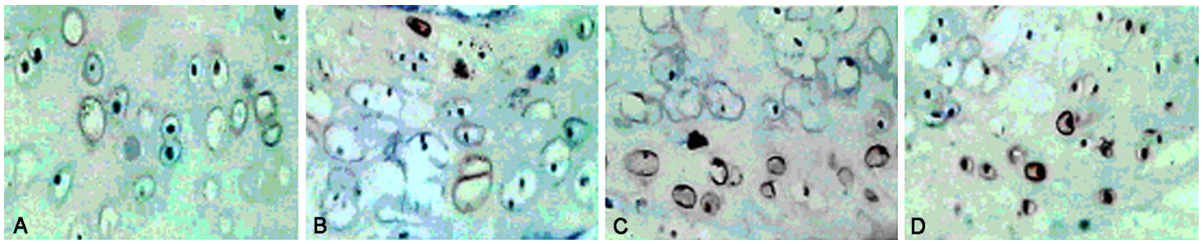

Fig. 1 IHC analysis of GSK-3β in the growth plate of wild-type P0 tibia. **a** Chondrocytes of the articular surface; **b** prehypertrophic chondrocytes; **c**, **d** hypertrophic chondrocytes

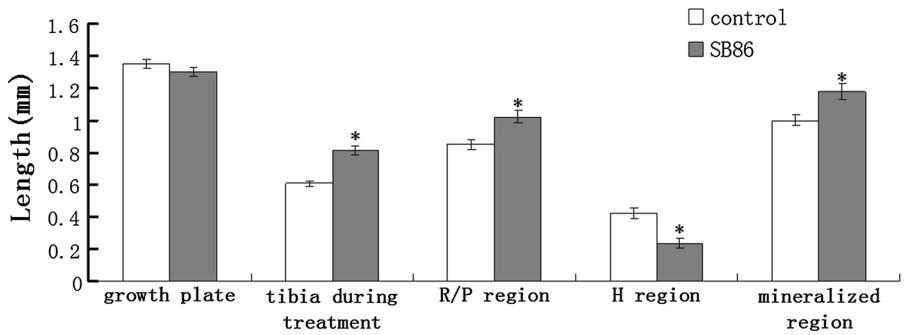

Fig. 2 Length of cultured tibias in control (treated with DMSO) and SB86 (treated with GSK-3 inhibitor) group. Proximal tibia growth plate (n = 9, $P = 0.362$); growth of the tibia during 6 days treatment (n = 9, $^*P = 0.00892$); resting and proliferating (R/P) region (n = 9, $^*P = 0.0251$); F, hypertrophic (H) region (n = 9, $^*P < 0.01$); mineralized region (n = 9, $^*P = 0.0365$). All data were compared using t test analysis, and significant differences are denoted by *asterisks* (n = 9, $^*P < 0.05$)

KO mice were generated. Efficiency of GSK-3β deletion was determined. As a result, long bone growth plate extracts from P0 mice demonstrated an 80 % reduction of GSK-3β in KO cartilage (Fig. 3a, b). In addition, IHC of P0 tibia paraffin section demonstrated strong expression of GSK-3β in the P/H zones of control mice, but exhibited significant loss of signals in KO mice (Fig. 3c). The expression of GSK-3β protein was also detected in calvaria tissue to evaluate the specificity of the deletion. As show in Fig. 3d, the GSK-3β expression was also affected. However, The cartilage-specific GSK-3β KO mice did not display any observable skeletal phenotype (Fig. 3e), and the length of the P0 tibias in KO mice was almost same compared with control (Fig. 3f).

β-Catenin is up-regulated through inhibition of GSK-3

The β-catenin was also detected after inhibition of GSK-3. As a result, an increase amount of β-catenin protein was observed specifically throughout the P/H zone of tibias in cultured organ treated with GSK-3 inhibitor (Fig. 4a, b). In addition, Western blot also showed significant increased β-catenin in the growth plate of cartilage-specific GSK-3β KO mice (Fig. 4c).

Discussion

The stabilization and reparation of cartilage is the major challenge in chronic joint diseases. In chronic joint diseases, including osteoarthritis (OA) and RA, progressive cartilage destruction is the dominant event responsible for the function loss, and it will lead to replacement surgery eventually (Williams et al. 2007). The underlying pathophysiologic events are various in RA and OA. For example, active infiltration and proteolytic digestion of cartilage is mainly accrue in RA (Fortier et al. 2010); reprogramming of chondrocytes triggered by signals of inflammation like IL-1 could found in both RA and OA (Fan et al. 2009); OA will arise when mechanical stress exceeding the physiologic capacity of self maintenance (Fioravanti

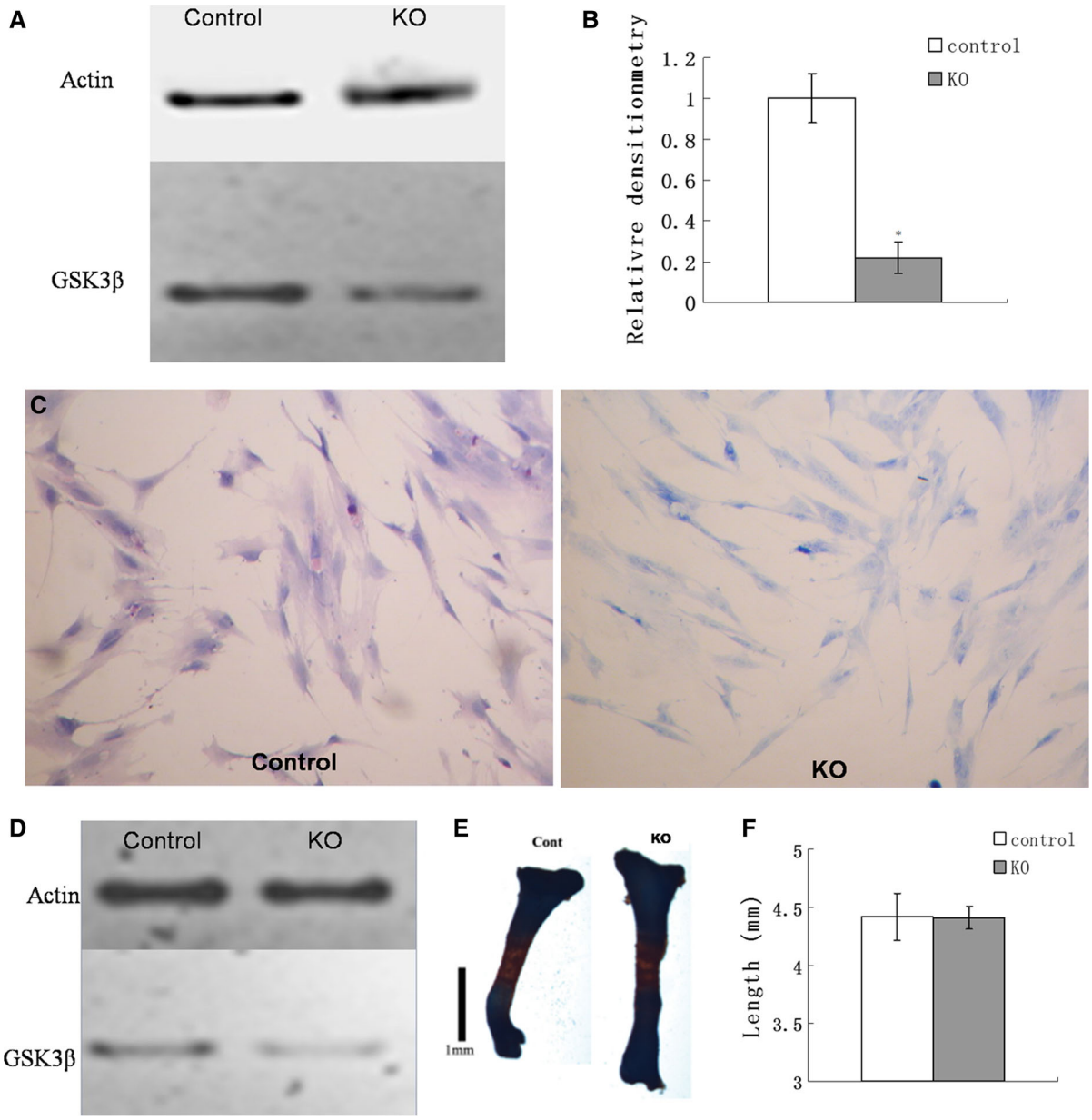

Fig. 3 Cartilage-specific GSK-3β mice (KO) were created and compared with the control. **a** Western blot analyses on growth plate extracts from newborn survival mice (P0) of KO and control mice; **b** densitometric quantification of GSK-3β protein in growth plate of P0 (n = 3, $*P < 0.03$); **c** IHC using GSK-3β antibodies on paraffin sections of P0 tibia; **d** Western blot analyses of GSK-3β protein in calvarial tissue; **e** tibias stained with alizarin red (bone) and alcian blue (cartilage) in control and KO mice; **e** Tibia length in control and KO P0 mice (n = 3, $P = 1.02$). All Western blot analyses used β-actin as a control. Quantification used *t* test analyses where significance was denoted with an *asterisk* (n = 3; $*P < 0.05$)

et al. 2010). As reported, molecular changes in chondrocyte behavior in both of these chronic joint diseases resemble the process of dedifferentiation in vitro during expansion of cartilage-derived primary chondrocytes (Im et al. 2010; Pulkkinen et al. 2012). Besides, several morphoses of the TGF-family were identified for their potential to enhance chondrocyte differentiation and cartilage maturation in artificial model of cartilage tissues (Nurminskaya et al. 2003). In this study, our results demonstrate the effects of

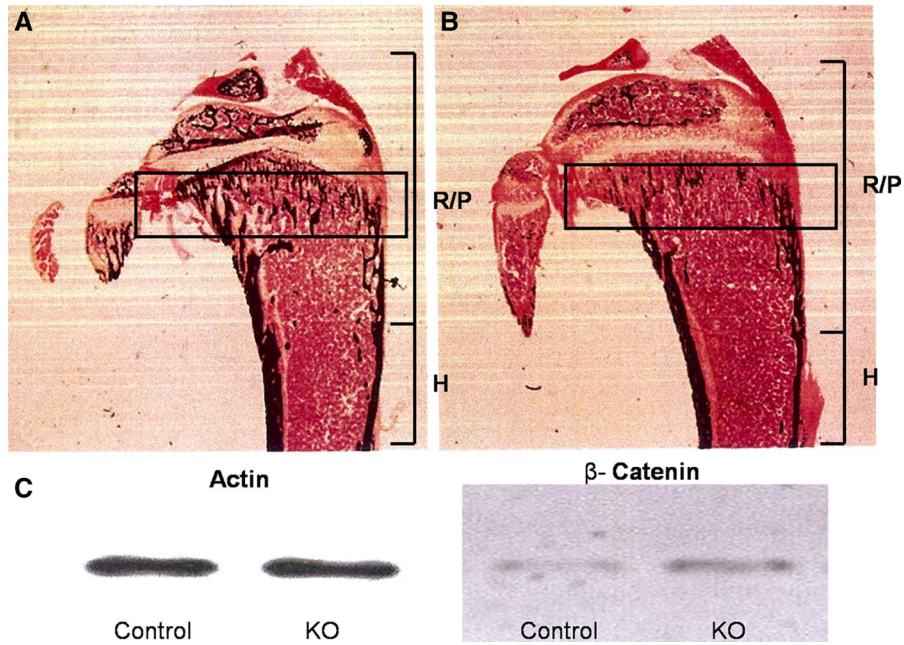

Fig. 4 IHC analysis of β-catenin in sections of tibia. **a** Control (treated with DMSO); **b** SB86 (treated with a GSK-3 inhibitor). *Black boxes* indicate the zone of β-catenin stained cells in prehypertrophic region. *Square brackets* indicate different regions of tibia. **c** Western blot analyses of β-catenin in P0 growth plate of KO and control mice. β-actin was used as a control

GSK-3β on bone growth in a tibia organ culture system. Inhibition of GSK-3β results in increased longitudinal growth of endochondral bones. This was likely caused by increased proliferation of chondrocytes in R/P regions. However, the length of mineralized zone was also relatively increased, this may suggest the processes in the growth plate (proliferation, differentiation, and replacement of cartilage by bone) occur at a fast rate upon GSK-3β inhibition, thus resulting in increased bone growth. In cartilage-specific Gsk3β KO mice, the expression of GSK-3β in cartilage is quite restricted. It is plausible that decreased GSK-3β expression in KO mice is due to decreased transcription, mRNA stability, or translation. However, the results can lead to alternative explanations, like the interactions between these alleles. Although it was reported that no cross talk between the two GSK-3β proteins has been described (Choi et al. 2009). Analyses of KO mice for both Gsk3β genes will be required to firmly establish redundant functions of these two genes. Beside, further studies were still needed to address the involved mechanisms.

As reported, GSK3-β regulate many cartilage-related genes, such as aggrecan and collagen (Reinhold et al. 2006; Kulkarni et al. 2007). Promotion and maintenance of the chondrocyte phenotype were operated through GSK3β expressing chondrocytes by inverting the collagen expression profile from type I collagen to type II collagen during prolonged cultivation in vitro. Interestingly, the expression of the large cartilage proteoglycan aggrecan was slightly down-regulated in chondrocytes transgenic for GSK3β (Maeda 2011). Additionally, GSK3-β were shown to maintain and promote later stages of chondrocyte differentiation rather than initiation of maturation, while BMPs promoted chondrocyte proliferation and inhibited terminal differentiation (Wang et al. 2010). In another research, addition of GSK3-β to short-term cultures of articular chondrocytes may promote redifferentiation in vitro by stimulation of the synthesis of type II collagen and proteoglycan expression (Majumdar et al. 2008). The expression of recombinant GSK3-β was stable in mixed cartilage tissues during 30 days of culture in vitro. Histochemical analysis revealed an increase of cartilage matrix production in GSK3-β transgenic cartilage tissues. In addition, the expression of GSK3-β in a subset of chondrocytes was sufficient to convert adjacent dedifferentiated chondrocytes into mature differentiated cartilage cells, as shown by the

switching of collagen synthesis from type I to type II collagen. Therefore, GSK3-β was a paracrine acting growth factor not only may stimulate matrix synthesis, but also may improve matrix quality of tissue-engineered cartilage tissue (Xu et al. 2012). In animal models of arthritis, severe and acute inflammation results in rapid joint destruction within a few weeks may extinguish possible anabolic effects of factors like BMPs (Canalis 2009). Since receptors of GSK3β were expressed in synovial tissue, GSK3-β may also exert a suppressive effect on pannus invasion in arthritic joints. As shown by Iwata et al., mesenchymal cell types derived from muscle or synovium may differentiate to cartilage upon stimulation with partially purified rabbit BMP, thus resembling human chondromatosis (Nogami et al. 2012). Although a mount of data imply a new therapeutic concept for chronic joint diseases by GSK3-β, application of GSK3-β was still critically when considering dosage and stimulation duration.

As reported, transcription factor β-catenin was a target of the canonical Wnt pathway and a central regulator of skeletal development (Dao et al. 2012). Inhibition of GSK-3 could accelerates the replacement of hypertrophic cartilage by mineralized tissue through Wnts signal (Steinert et al. 2012; Foldager et al. 2011). In this study, β-Catenin is up-regulated through inhibition of GSK-3β. Although chondrocyte function is very sensitive to both supra-physiological and super-physiological levels of β-catenin, the moderate change appears to induce changes in cartilage development and bone growth. However, this result only demonstrate the relationship between GSK-3β and Wnt/β-catenin signaling. It will be interesting to examine in the future whether increased β-catenin or other aspects of cartilage-specific loss of GSK-3β alters the susceptibility to cartilage degeneration in osteoarthritis.

Acknowledgments This study was supported by Grants from Leading Academic Discipline Project of State Administration of Traditional Chinese Medicine of China, Leading Academic Discipline Project of Shanghai, China (No. T0303) and Shanghai Education Commission research projects (No. 2011JW60) and research projects of PU-TUO hospital (No. 2013QK20).

References

Baron R, Rawadi G (2007) Targeting the Wnt/β-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 148(6):2635–2643

Bhukhai K, Suksen K, Bhummaphan N, Janjorn K, Thongon N, Tantikanlayaporn D, Piyachaturawat P, Suksamrarn A, Chairoungdua A (2012) A phytoestrogen diarylheptanoid mediates estrogen receptor/Akt/glycogen synthase kinase 3β protein-dependent activation of the Wnt/β-catenin signaling pathway. J Biol Chem 287(43):36168–36178

Boku S, Nakagawa S, Masuda T, Nishikawa H, Kato A, Kitaichi Y, Inoue T, Koyama T (2009) Glucocorticoids and lithium reciprocally regulate the proliferation of adult dentate gyrus-derived neural precursor cells through GSK-3beta and beta-catenin/TCF pathway. Neuropsychopharmacology 34(3):805–815. doi:10.1038/npp.2008.198

Canalis E (2009) Growth factor control of bone mass. J Cell Biochem 108(4):769–777

Choi Y, Kang SS, Jin EJ (2009) BMP-2 treatment of C3H10T1/2 mesenchymal cells blocks MMP-9 activity during chondrocyte commitment. Cell Biol Int 33(8):887–892

Dao DY, Jonason JH, Zhang Y, Hsu W, Chen D, Hilton MJ, O'Keefe RJ (2012) Cartilage-specific β-catenin signaling regulates chondrocyte maturation, generation of ossification centers, and perichondrial bone formation during skeletal development. J Bone Miner Res 27(8):1680–1694

Day TF, Guo X, Garrett-Beal L, Yang Y (2005) Wnt/β-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8(5):739–750

Fan Z, Tardif G, Hum D, Duval N, Pelletier J-P, Martel-Pelletier J (2009) Hsp90β and p130cas: novel regulatory factors of MMP-13 expression in human osteoarthritic chondrocytes. Ann Rheum Dis 68(6):976–982

Fioravanti A, Collodel G, Petraglia A, Nerucci F, Moretti E, Galeazzi M (2010) Effect of hydrostatic pressure of various magnitudes on osteoarthritic chondrocytes exposed to IL-1β. Indian J Med Res 132:209–217

Foldager CB, Nielsen AB, Munir S, Ulrich-Vinther M, Søballe K, Bünger C, Lind M (2011) Combined 3D and hypoxic culture improves cartilage-specific gene expression in human chondrocytes. Acta Orthop 82(2):234–240

Fortier LA, Motta T, Greenwald RA, Divers TJ, Mayr KG (2010) Synoviocytes are more sensitive than cartilage to the effects of minocycline and doxycycline on IL-1α and MMP-13-induced catabolic gene responses. J Orthop Res 28(4):522–528

Hill TP, Später D, Taketo MM, Birchmeier W, Hartmann C (2005) Canonical Wnt/β-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 8(5):727–738

Im HJ, Kim JS, Li X, Kotwal N, Sumner DR, van Wijnen AJ, Davis FJ, Yan D, Levine B, Henry JL (2010) Alteration of sensory neurons and spinal response to an experimental osteoarthritis pain model. Arthritis Rheum 62(10):2995–3005

Kulkarni NH, Wei T, Kumar A, Dow ER, Stewart TR, Shou J, N'cho M, Sterchi DL, Gitter BD, Higgs RE (2007) Changes in osteoblast, chondrocyte, and adipocyte lineages mediate the bone anabolic actions of PTH and small molecule GSK-3 inhibitor. J Cell Biochem 102(6):1504–1518

Lewis R, Feetham CH, Barrett-Jolley R (2011) Cell volume regulation in chondrocytes. Cell Physiol Biochem 28(6):1111–1122

Maeda S (2011) An impact of CCN2–BMP-2 complex upon chondrocyte biology: evoking a signalling pathway bypasses ERK and Smads? J Biochem 150(3):219–221

Majumdar MK, Chockalingam PS, Bhat RA, Sheldon R, Keohan C, Blanchet T, Glasson S, Morris EA (2008) Immortalized mouse articular cartilage cell lines retain chondrocyte phenotype and respond to both anabolic factor BMP-2 and pro-inflammatory factor IL-1. J Cell Physiol 215(1):68–76

Moon RT, Bowerman B, Boutros M, Perrimon N (2002) The promise and perils of Wnt signaling through β-catenin. Science 296(5573):1644–1646

Naves MA, Requião-Moura LR, Soares MF, Silva-Júnior JA, Mastroianni-Kirsztajn G, Teixeira V (2011) Podocyte Wnt/ß-catenin pathway is activated by integrin-linked kinase in clinical and experimental focal segmental glomerulosclerosis. J Nephrol 25(3):401–409

Nogami M, Tsuno H, Koike C, Okabe M, Yoshida T, Seki S, Matsui Y, Kimura T, Nikaido T (2012) Isolation and characterization of human amniotic mesenchymal stem cells and their chondrogenic differentiation. Transplantation 93(12):1221–1228

Nurminskaya M, Magee C, Faverman L, Linsenmayer TF (2003) Chondrocyte-derived transglutaminase promotes maturation of preosteoblasts in periosteal bone. Dev Biol 263(1):139–152

Oseni AO, Butler PE, Seifalian AM (2013) Optimization of chondrocyte isolation and characterization for large-scale cartilage tissue engineering. J Surg Res 181(1):41–48

Park S-I, Lee H-R, Kim S, Ahn M-W, Do SH (2012) Time-sequential modulation in expression of growth factors from platelet-rich plasma (PRP) on the chondrocyte cultures. Mol Cell Biochem 361(1–2):9–17

Pasco MY, Catoire H, Parker JA, Brais B, Rouleau GA, Néri C (2010) Cross-talk between canonical Wnt signaling and the sirtuin-FoxO longevity pathway to protect against muscular pathology induced by mutant PABPN1 expression in *C. elegans*. Neurobiol Dis 38(3):425–433

Primot A, Baratte B, Gompel M, Borgne A, Liabeuf S, Romette J-L, Jho E-H, Costantini F, Meijer L (2000) Purification of GSK-3 by affinity chromatography on immobilized axin. Protein Expr Purif 20(3):394–404

Pulkkinen HJ, Tiitu V, Valonen P, Jurvelin J, Rieppo L, Töyräs J, Silvast T, Lammi M, Kiviranta I (2012) Repair of osteochondral defects with recombinant human type II collagen gel and autologous chondrocytes in rabbit. Osteoarthr Cartil 21(3):481–490

Reinhold MI, Kapadia RM, Liao Z, Naski MC (2006) The Wnt-inducible transcription factor Twist1 inhibits chondrogenesis. J Biol Chem 281(3):1381–1388

Steinert AF, Rackwitz L, Gilbert F, Nöth U, Tuan RS (2012) Concise review: the clinical application of mesenchymal stem cells for musculoskeletal regeneration: current status and perspectives. Stem Cells Transl Med 1(3):237–247

Studer D, Millan C, Ozturk E, Maniura-Weber K, Zenobi-Wong M (2012) Molecular and biophysical mechanisms regulating hypertrophic differentiation in chondrocytes and mesenchymal stem cells. Eur Cell Mater 24(24):118–135

Taipaleenmäki H, Abdallah BM, AlDahmash A, Säämänen A-M, Kassem M (2011) Wnt signalling mediates the cross-talk between bone marrow derived pre-adipocytic and pre-osteoblastic cell populations. Exp Cell Res 317(6):745–756

Toh WS, Lee EH, Guo X-M, Chan JK, Yeow CH, Choo AB, Cao T (2010) Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. Biomaterials 31(27):6968–6980

Wai PY, Kuo PC (2008) Osteopontin: regulation in tumor metastasis. Cancer Metastasis Rev 27(1):103–118

Wang L, Shao YY, Ballock RT (2010) Thyroid hormone-mediated growth and differentiation of growth plate chondrocytes involves IGF-1 modulation of β-catenin signaling. J Bone Miner Res 25(5):1138–1146

Williams J Jr, Ragland P, Clarke S (2007) Constrained components for the unstable hip following total hip arthroplasty: a literature review. Int Orthop 31(3):273–277

Xiao Z, Zhang S, Cao L, Qiu N, David V, Quarles LD (2010) Conditional disruption of Pkd1 in osteoblasts results in osteopenia due to direct impairment of bone formation. J Biol Chem 285(2):1177–1187

Xu T, Wu M, Feng J, Lin X, Gu Z (2012) RhoA/Rho kinase signaling regulates transforming growth factor-β1-induced chondrogenesis and actin organization of synovium-derived mesenchymal stem cells through interaction with the Smad pathway. Int J Mol Med 30(5):1119