

# Glucocorticoid Signaling and Bone Biology

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**Key words**

- apoptosis
- autophagy
- wnt
- osteoblast
- osteoclast
- OPG

**Abstract**

Since glucocorticoids remain an effective therapeutic option for the treatment of many inflammatory and autoimmune diseases, glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis. Fractures may occur in as many as 30–50% of patients receiving chronic glucocorticoid therapy. Under physiological conditions, glucocorticoids are required for normal bone development due to their regulation of osteoblast differentiation, possibly via the Wnt/ $\beta$ -catenin pathway and TSC22D3. However, serum levels of endogenous corticosterone are elevated in aged mice and glucocorticoids exert negative effects on the survival of osteoblasts and osteocytes as well as angiogenesis. Glucocorticoid treatments impair bone formation and enhance bone resorption. Excess glucocorticoids induce osteoblast and osteocyte apoptosis by increasing pro-apoptotic molecules, reactive oxygen species, and endoplasmic reticulum stress and suppressing the Wnt/ $\beta$ -catenin path-

way. Autophagy protects osteocytes from glucocorticoid-induced apoptosis, but passed some threshold, the process of autophagy leads the cells to apoptosis. Excess glucocorticoids impair osteoblastogenesis by inducing Wnt antagonists, including Dkk1, Sost, and sFRP-1. However, the findings are controversial and the involvement of Wnt antagonists requires further study. Excess glucocorticoids reduce the phosphorylation of Akt and GSK3 $\beta$ , which enhances the degradation of  $\beta$ -catenin. Excess glucocorticoids have been shown to modulate the expression of miRNAs, including miR-29a, miR-34a-5p, and miR-199a-5p, which regulate the proliferation and differentiation of osteoblast lineage cells. Excess glucocorticoids also enhance bone resorption by reducing OPG expression, increasing Rankl expression and reactive oxygen species, and prolonging the life span of osteoclasts; however, they also suppress the bone-degrading capacity of osteoclasts by disturbing the organization of the cytoskeleton.

received 11.04.2016  
accepted 08.06.2016

**Bibliography**

DOI <http://dx.doi.org/10.1055/s-0042-110571>  
Horm Metab Res 2016;  
48: 755–763  
© Georg Thieme Verlag KG  
Stuttgart · New York  
ISSN 0018-5043

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**Introduction**

Glucocorticoid (GC)-induced osteoporosis is the most common form of secondary osteoporosis. In humans treated with GCs, an early rapid decline in bone mineral density, possibly caused by enhanced bone resorption, is followed by a slower progressive decline in bone mineral density, which is due to reduced bone formation. Cancellous bone is preferentially lost in GC-induced osteoporosis, and fractures frequently occur at sites enriched in cancellous bone, such as the vertebrae and femoral neck [1]. Excess GCs are also associated with the early activation of genes associated with osteoclastogenesis and the later suppression of genes associated with osteogenesis and mineralization in mice [2]. A major

issue in rodent models of GC-induced osteoporosis is that the loss of cancellous bone is not consistently observed in rodents. GC treatments in large animals, including rabbits and sheep, show a similar phenotype to that in humans [3]. GC receptor (GR, official symbol Nr3c1) forms a complex containing chaperone molecules including HSP90 in the cytoplasm. Ligand binding to GR results in the dissociation of the complex and GR translocates into the nucleus. The GR homodimer directly binds GC responsive elements (GREs) in DNA and upregulates or suppresses the transcription of associated genes. Monomeric GR is also involved in the regulation of gene transcription. It binds to transcription factors by protein-protein interactions and promotes or inhibits gene transcription [4].

The physiological functions of GCs on bone, the pathological effects of excess GCs on bone, and the pathogenesis of GC-induced osteoporosis in osteoblast and osteoclast lineages are described here.

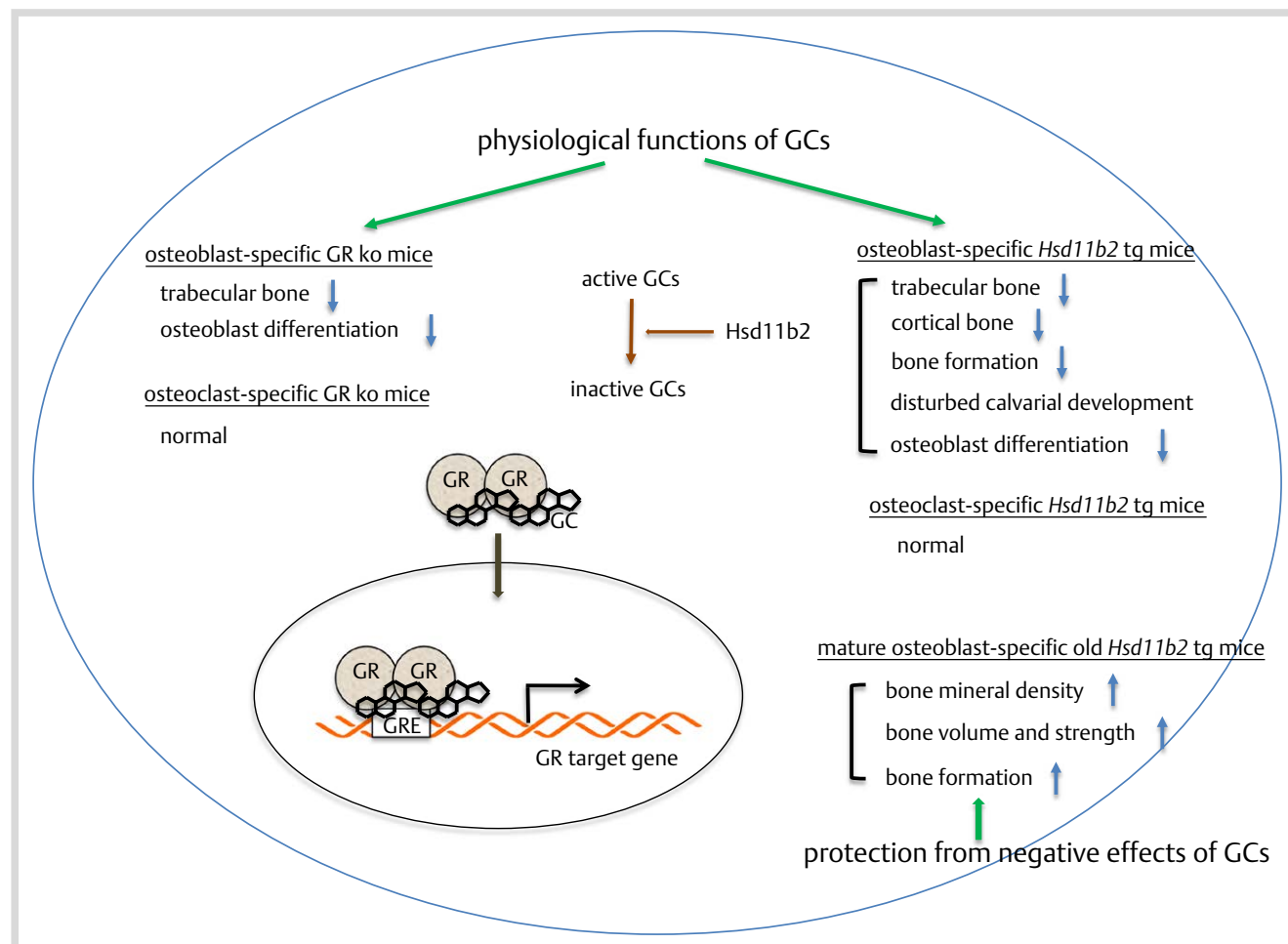
### Physiological Functions of GCs in Bone

The physiological functions of GR signaling in bone were examined using 2 methods (◻ Fig. 1). In some tissues, GCs are locally metabolized before receptor binding by 2 enzymes: the inactivating enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (Hsd11b2), which converts biologically active GCs to their inert 11-keto metabolites, and the activating enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (Hsd11b1), which works in the opposite manner. In order to inhibit the effects of GCs, human or rat *HSD11B2* cDNA was overexpressed in various differentiation stages of osteoblast lineage cells or in osteoclasts.

In *Hsd11b2* transgenic mice under the control of the 2.3-kb rat *Col1a1* promoter, which directs transgene expression to immature and mature osteoblasts, the trabecular bone volume in the lumbar vertebrae in female mice and cortical thickness in both sexes are less than those in wild-type mice. Furthermore, osteoblast differentiation was found to be impaired in primary osteoblasts derived from *Hsd11b2* transgenic mice [5,6] (◻ Fig. 1).

Another group also analyzed the same *Hsd11b2* transgenic line. Calvarial development was disturbed due to reduced bone formation, ectopic cartilage formation in the sagittal suture, and a defect in the postnatal removal of parietal cartilage (◻ Fig. 1). These phenotypes were explained by a reduction in Wnt expression in osteoblasts, which leads to impaired osteoblast differentiation and a delay in cranial cartilage degradation by *Mmp14*, which is reduced in *Hsd11b2* transgenic mice [7]. The same group showed significant reductions in the trabecular bone of the vertebrae and tibiae in *Hsd11b2* transgenic mice of both sexes [8]. Similar phenotypes were also observed in *Hsd11b2* transgenic mice under the control of the 3.6-kb rat *Col1a1* promoter, which directs transgene expression in osteoblast precursors, immature osteoblasts, and mature osteoblasts [9].

GR-deficient (GR<sup>-/-</sup>) mice die just after birth due to atelectasis [10]. The skeletal development of GR<sup>-/-</sup> mice during the embryonic stage is similar to that of wild-type mice [11]. Since calvarial development is disturbed from the embryonic stage in *Hsd11b2* transgenic mice under the control of the 2.3-kb *Col1a1* promoter [7], ligand inactivation and GR deletion may cause different phenotypes. Osteoblast lineage-specific GR conditional knockout mice using *Runx2* Cre transgenic mice show reductions in the trabecular bone mass, and the differentiation of primary osteoblasts from GR conditional knockout mice is impaired [11] (◻ Fig. 1). These findings indicate that GC is required for



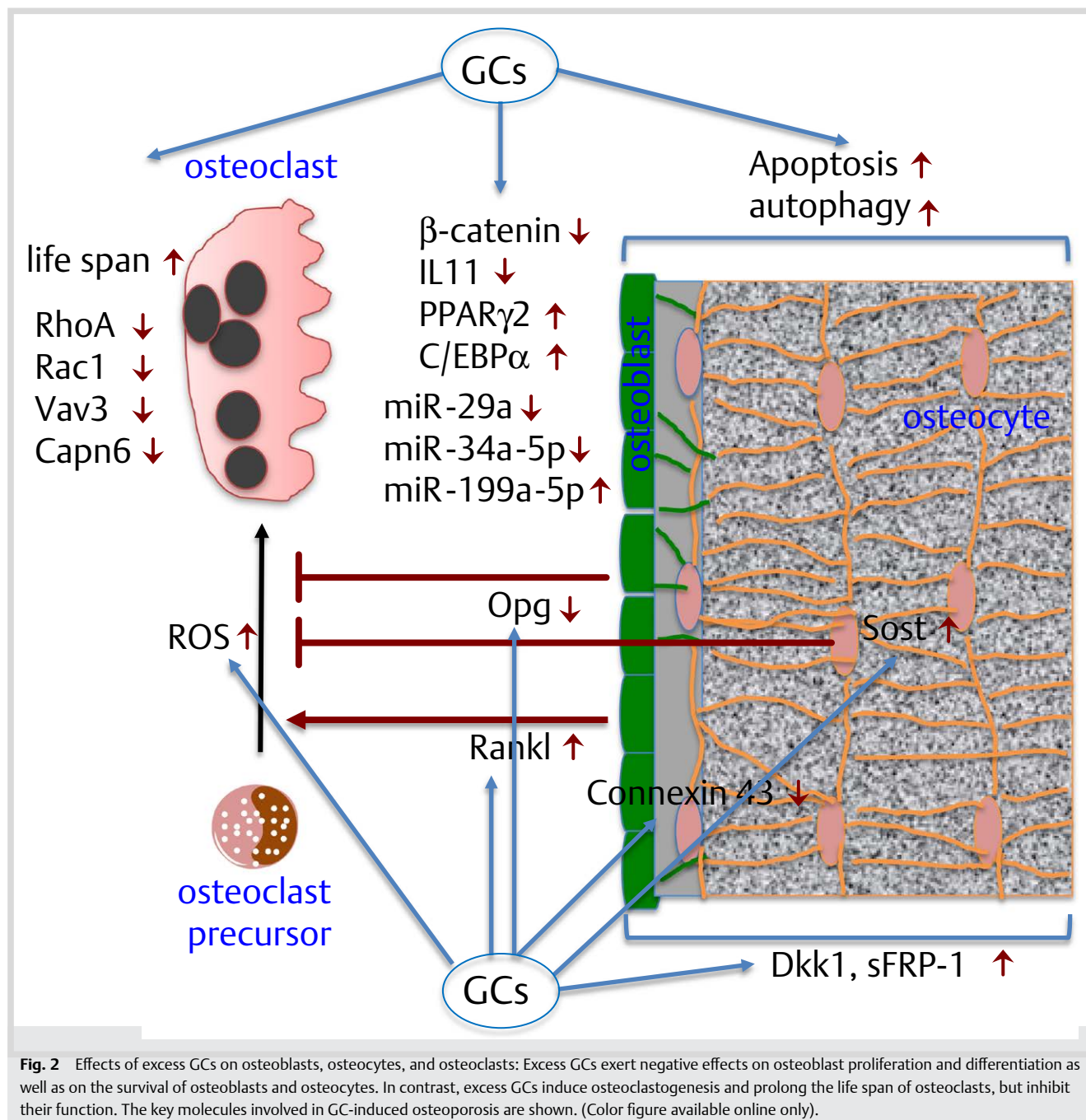
**Fig. 1** Physiological functions of GCs: Physiological functions of GCs were examined using osteoblast-specific *Hsd11b2* transgenic (tg) mice, osteoclast-specific *Hsd11b2* tg mice, osteoblast-specific GR knockout (ko) mice, and osteoclast-specific GR ko mice. GC signaling physiologically enhances bone formation, whereas inactivation of GCs in mature osteoblasts in old mice increases bone formation due to the protection from the negative effects of GCs. (Color figure available online only).

normal bone development and osteoblast differentiation under physiological conditions.

*HSD11B2* transgenic mice under the control of the *osteocalcin* (*Bglap2*) promoter, which directs transgene expression in mature osteoblasts, show similar bone density, strength, and histomorphometric parameters to those in wild-type mice at 5 months of age [12]. The serum level of endogenous corticosterone is higher in 31-month-old wild-type mice than in 4-month-old wild-type mice, and bone mineral density, bone volume and strength, and bone formation are higher in *HSD11B2* transgenic mice than in wild-type mice at 21 months of age. The increases observed in aged *HSD11B2* transgenic mice appear to be attributed to the protection from the negative effects of GCs on the survival of osteoblasts and osteocytes as well as angiogenesis [13] (◻ Fig. 1). Thus, GCs exert negative effects on the maintenance of bone in aged mice.

In *Hsd11b2* transgenic mice under the control of the tartrate-resistant acid phosphatase (Trap) promoter, which directs transgene expression to osteoclasts, bone mineral density and histomorphometric parameters were found to be similar to those in wild-type mice [14]. Furthermore, the osteoclast-lineage deletion of GR using *Ly2* Cre knock-in mice does not cause any abnormalities under physiological conditions [15]. Therefore, GC signaling has no significant effect on osteoclasts under physiological conditions.

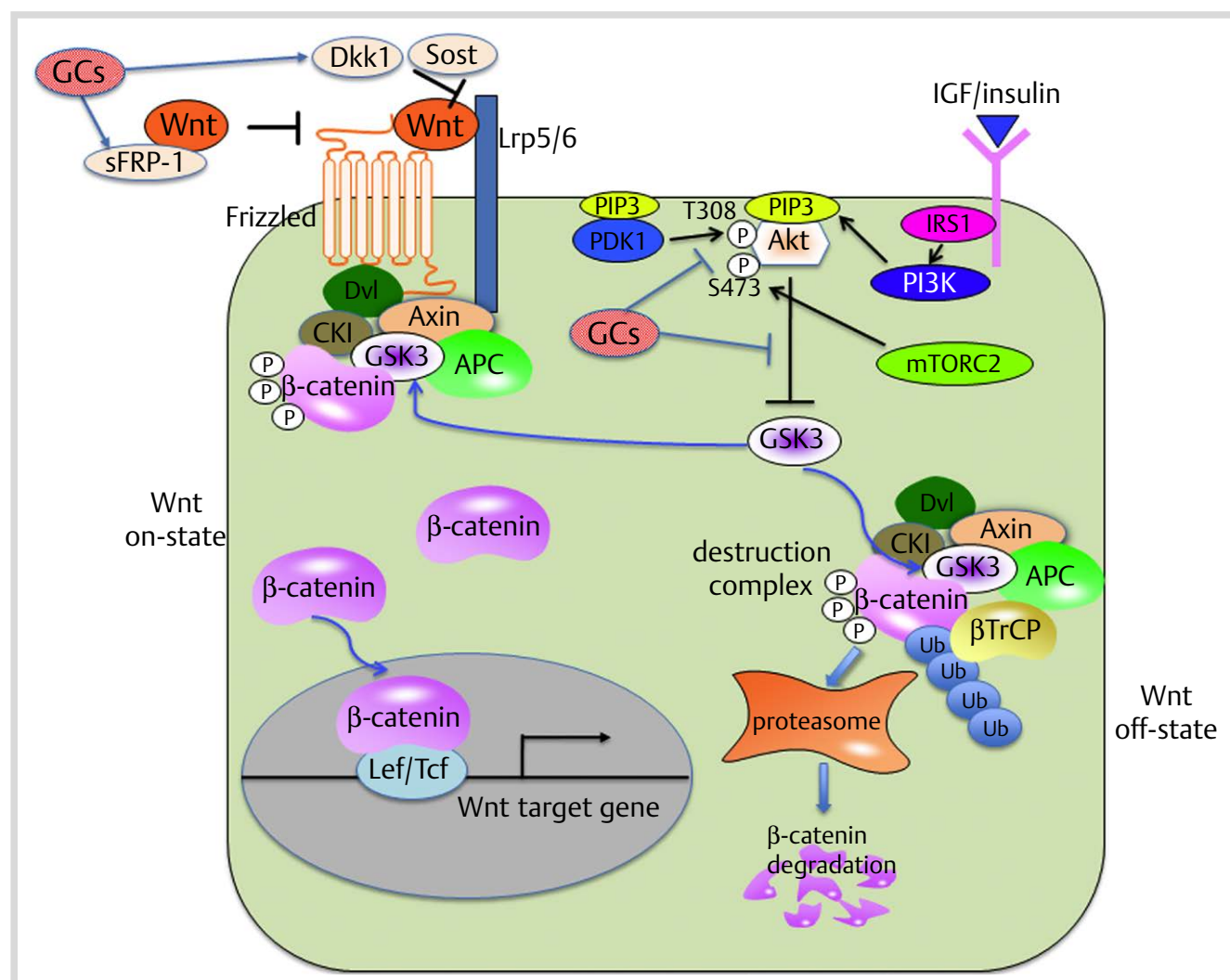
Some reports suggested the mechanisms for the positive effects of GCs on osteoblasts. The overexpression of *TSC22D3*, also called the GC-induced leucine zipper (*GILZ*) that encodes a GC anti-inflammatory effect mediator, under the control of the 3.6-kb rat *Col1a1* promoter increases bone mass due to enhanced bone formation. *TSC22D3* interacts with C/EBPs and disrupts C/EBP-mediated *Pparγ2* gene transcription [16]. Therefore, GCs may



enhance osteoblast differentiation through TSC22D3, which inhibits mesenchymal stem cell differentiation into adipocytes. Dexamethasone (Dex) is used to enhance osteoblast differentiation *in vitro*; however, the underlying mechanisms have not yet been elucidated in detail. Dex was recently shown to stimulate the expression of fibronectin, integrin  $\alpha 5$ , and serum-GC-induced protein kinase 1 (SGK1), which induces Sec5-GEF-H1 interactions. Enhanced Sec5-GEF-H1 interactions promote GEF-H1 to adhesion sites, which leads to the attachment of fibronectin to integrin at adhesion sites in order to generate cellular tension. These processes are required for the induction of osteoblast differentiation by Dex *in vitro* [17].

### Apoptosis Induced by Excess GCs

The administration of prednisolone (2.1 mg/kg/d) for 27 days to mice has been shown to increase osteoblast apoptosis in the vertebrae by 3-fold and the frequency of osteocyte apoptosis in metaphysical cortical bone reached 28%, resulting in a decrease in vertebral cancellous bone due to reduced bone formation [18]. Bisphosphonates and calcitonin prevent GC-induced apoptosis in osteoblasts and osteocytes [19]. The administration of GCs similarly reduces bone mass in wild-type mice and *Bglap2*-promoter-*HSD11B2* transgenic mice; however, the apoptosis of osteoblasts and osteocytes is induced in wild-type mice, but not in transgenic mice and vertebral compression strength is lost in GC-treated wild-type mice, but not in the transgenic mice [12] (Fig. 2). Osteoblast apoptosis is prevented in *GR*<sup>-/-</sup> mice, but not in GR-dimerization-defective mice, indicating that GR dimerization is not required for osteoblast apoptosis induced by



**Fig. 3** Wnt signaling and PI3K-Akt signaling pathways: The binding of Wnt ligands to Fz and LRP5/6 leads to the membrane recruitment of the destruction complex, including Axin, APC, GSK3, CKI, and  $\beta$ -catenin, but not  $\beta$ TrCP by Dvl, which results in the inhibition of  $\beta$ -catenin ubiquitination and degradation. The destruction complex without  $\beta$ TrCP is saturated with phosphorylated  $\beta$ -catenin, and newly formed  $\beta$ -catenin freely translocates to the nucleus, binds to Lef/Tcf transcription factors, and induces the transcription of Wnt target genes. Dkk1 and Sost bind LRP5/6 and inhibit Fz-LRP5/6 complex formation. sFRP-1 binds to Wnts

and inhibits binding to the receptors. In the absence of Wnt ligand binding, the destruction complex with  $\beta$ TrCP ubiquitinates  $\beta$ -catenin, which is then degraded in the proteasome. The destruction complex is recycled for  $\beta$ -catenin ubiquitination. Akt is phosphorylated by PDK1 and mTORC2, and phosphorylated (activated) Akt inhibits GSK3 by phosphorylation. Phosphorylated (inactivated) GSK3 loses the ability to phosphorylate  $\beta$ -catenin. Since the phosphorylation of  $\beta$ -catenin is required for  $\beta$ -catenin ubiquitination by  $\beta$ TrCP, the degradation of  $\beta$ -catenin is inhibited. (Color figure available online only).



GCs [11]. A number of mechanisms have been reported for GC-induced apoptosis. Excess GCs have been shown to increase the pro-apoptotic factors, Bim and Bak, and decrease the pro-survival factor, BclXL in vitro [20,21]. GCs induce the expression of E4bp4, which is a basic leucine zipper transcription factor, and upregulate Bim through E4bp4 [22]. Excess GCs also upregulate p53 protein levels, resulting in increases in p21, Noxa, and Puma [23]. Excess GCs activate Pyk2 by inducing  $Ca^{2+}$  entrance from the extracellular space, and the activated Pyk2 induces JNK activation followed by cell detachment and apoptosis [24]. Excess GCs increase reactive oxygen species (ROS), which induce apoptosis through the PKC $\beta$ /p66shc/JNK pathway, while excess GCs inhibit Akt, resulting in the suppression of the Wnt/ $\beta$ -catenin pathway, which is required for osteoblastogenesis and the prevention of cell death [25]. Ccl7, which is a chemotactic myokine, activates the  $\beta$ -catenin pathway through the phosphorylation of glycogen synthase kinase 3 (GSK-3), and exerts protective effects against the death of osteocyte-like MLO-Y4 cells induced by GCs [26]. Endoplasmic reticulum (ER) stress is alleviated by the phosphorylation of eIF2a, and inhibitors of eIF2a dephosphorylation have been shown to prevent GC-induced apoptosis in osteoblasts and osteocytes, indicating that the proapoptotic action of GCs is mediated, in part, through ER stress [27].

### Autophagy Induced by Excess GCs

A GC treatment increases the expression of the markers for autophagy in osteocytes (Fig. 2). When autophagy is inhibited, osteocyte numbers are decreased further by a GC treatment, suggesting that autophagy is a self-protective process in osteocytes in response to excess GCs [28]. Furthermore, the GC dosage determines osteocyte cell fate: a low dose of GCs induces autophagy while a high dose induces apoptosis [29]. Atg7 is involved in the formation of autophagosomes and is required for autophagy. Conditional Atg7 knockout mice in osteocytes using *Dmp1* Cre transgenic mice show reduced osteoblast numbers and bone formation in cancellous bone; however, these are not reduced further by a GC treatment. Osteocyte density is also known to be reduced in conditional Atg7 knockout mice under physiological conditions, but is not reduced further by a treatment with GCs, indicating that the suppression of autophagy in osteocytes does not worsen the negative effects of GCs. These findings suggest that the process of autophagy is required for osteocyte death induced by GCs [30]. Therefore, autophagy appears to protect osteocytes from the negative effects of GCs; however, when these effects reach a certain threshold, the process of autophagy leads cells to apoptosis.

### The Role of Wnt Antagonists in GC-Induced Osteoporosis

The binding of Wnt ligands to their receptors frizzled (Fz) and LRP5 or LRP6 leads to the membrane recruitment of axin proteins by dishevelled (Dvl), which ultimately results in the inhibition of  $\beta$ -catenin ubiquitination and degradation (Fig. 3). As a mechanism responsible for GC-induced reductions in bone formation, the modulated expression of Wnt antagonists has been extensively examined. Dkk1, which disrupts Wnt-induced Fz-Lrp5/6 complex formation, is upregulated by Dex in primary cultured human osteoblasts, and Dex induces the reporter activity

of the Dkk1 promoter [31,32]. Furthermore, the suppression of Dkk1 using antisense oligonucleotides or siRNA for Dkk1 abrogates the inhibition of osteoblast differentiation by GCs [33,34]. A Dex treatment in osteoblastic MC3T3-E1 cells induces the bone morphogenetic protein (BMP) antagonists follistatin and Dan, the Wnt antagonist, sFRP-1, and the Wnt signal inhibitor, Axin-2 [35] (Fig. 3). However, GC-treated mice show variable results for the expression of Wnt antagonists. The expression of Dkk1 and Sost, which also disrupts Wnt-induced Fz-Lrp5/6 complex formation, is reduced by a prednisolone treatment for 7 days; however, after the treatment for 56 days, Dkk1 expression is increased while that of Sost returns to the level before the treatment [2]. Dkk1 expression is upregulated by a prednisolone treatment for 4 weeks [36]. A Dkk1 antisense treatment for 5 weeks was found to alleviate Dex-induced bone loss [33]. The treatment with prednisolone for 28 days increases Sost expression in wild-type mice at 5 months of age and reduces bone mass in wild-type mice but not in *Sost*<sup>-/-</sup> mice. However, the reduction in bone formation and the increase in apoptosis are similarly observed between wild-type and *Sost*<sup>-/-</sup> mice after the GC treatment [37]. In contrast, the protein expression of Dkk1 and Sost is reduced in bone marrow supernatants from wild-type mice after a GC treatment [30]. We also observed a reduction in Dkk1 and Sost mRNA levels in the osteocyte fraction in the mice at 12 weeks of age after a treatment with Dex for 4 weeks (unpublished observation). In humans, the expression of Dkk1 is decreased by short- and long-term treatments with GCs, while that of Sost only increases after the long-term treatment [38]. Therefore, the duration of GC treatment and the age of the animals may affect the response of Sost expression to excess GCs. Although Dkk1 is upregulated in human primary osteoblasts by a treatment with Dex, this upregulation is observed for 1–48 h [31,32]. In a primary culture of GFP-positive osteoblasts from 2.3-kb *Col1a1* promoter GFP transgenic mice, Dkk1 and sFRP-1 are upregulated by a treatment with corticosterone for 1 and 0.5 h, respectively, but return to control levels after 2 h [39]. These findings indicate that the upregulation of Dkk1 and sFRP-1 in vitro may be transient. Thus, the involvement of Dkk1 and other Wnt antagonists in the pathogenesis of GC-induced osteoporosis is still controversial and, thus, requires further study.

### Effects of Excess GCs through the Modulation of GSK3 $\beta$

Dex reduces the phosphorylation of GSK3 $\beta$  and amount of nuclear  $\beta$ -catenin in MC3T3-E1 cells, and a treatment with the GSK3 inhibitor, 6-bromindirubin-3'-oxime (BIO), mostly alleviates the negative effects of Dex in the bone of mice [40]. Furthermore, Dex inhibits Akt phosphorylation in mouse and human primary osteoblasts and osteoblastic MC3T3-E1 cells and also decreases  $\beta$ -catenin/TCF-dependent transcription in a GSK3-dependent manner in MC3T3-E1 cells [41]. There are 2 glycogen synthase kinase 3 (GSK3) proteins, GSK3 $\alpha$  and GSK3 $\beta$ . Three major pools of GSK3 exist under basal conditions: (1) GSK3 is part of the Wnt signaling complex; (2) GSK3 is part of the hedgehog signaling complex, which consists of Cos2, Gli, and other proteins; and (3) there is a free pool of GSK3. In Wnt signaling, GSK3 $\beta$  is a component of the destruction complex, which is composed of Axin, APC, CK1, and GSK3 $\beta$ . When Fz-LRP5/6 receptors are not engaged, CK1 and GSK3 $\beta$  sequentially phosphorylate Axin-bound  $\beta$ -catenin. Phosphorylated  $\beta$ -catenin is recognized

by  $\beta$ -TrCP, part of an E3 ubiquitin ligase complex, and the ubiquitinated  $\beta$ -catenin is rapidly degraded by proteasome. The IGF-IGF receptor-PI3K-Akt pathway plays key roles in skeletal growth and endochondral ossification [42] (○ Fig. 3). Akt phosphorylates Ser21 in GSK3 $\alpha$  and Ser9 in GSK3 $\beta$ , which inactivates them. Contradictory findings have been reported for the involvement of Akt in Wnt signaling. Insulin and IGF have been shown to activate  $\beta$ -catenin/TCF-dependent transcription, mutant PI3K and kinase-dead Akt inhibited the  $\beta$ -catenin/TCF-dependent transcription induced by insulin and IGF, and constitutively active PI3K and Akt activated  $\beta$ -catenin/TCF-dependent transcription in a HepG2 hepatoma cell line [43]. Wnt was found to increase the phosphorylation of Akt; and phosphorylated (activated) Akt bound to the Axin-GSK3 $\beta$  complex in the presence of Dvl, phosphorylated GSK3 $\beta$ , and increased free  $\beta$ -catenin in PC12 pheochromocytoma cells [44]. In contrast, Wnt failed to phosphorylate Akt, the introduction of kinase-dead GSK3 $\beta$  failed to activate  $\beta$ -catenin/TCF-dependent transcription in human embryonic kidney 293 cells, and insulin exhibited a limited ability to increase the Ser9 phosphorylation of Axin-2-bound GSK3 $\beta$  in 293 cells and CHOIR, a Chinese hamster ovary cell line stably expressing human insulin receptors [45]. Although these experiments were performed using similar methods, the findings obtained were contradictory. The activation of the Wnt pathway by PI3K-Akt signaling appears to be dependent on the cells used in the experiments. The physiological importance of the phosphorylation levels of GSK3 in Wnt signaling is also a matter of debate. In double homozygous knock-in mice in which GSK3 $\alpha$  (Ser21) and GSK3 $\beta$  (Ser9) were both changed to Ala, GSK3 $\alpha$  and GSK3 $\beta$  are constitutively active. However,  $\beta$ -catenin is increased and  $\beta$ -catenin/TCF-dependent transcription is activated under the stimulation of Wnt3a in GSK3 $\alpha$ /GSK3 $\beta$  homozygous knock-in ES cells at similar levels to those in wild-type cells [46]. In a recently proposed model, the Wnt receptor-ligand interaction is shown to induce the dissociation of  $\beta$ -TrCP from the destruction complex, leaving phosphorylated  $\beta$ -catenin in the destruction complex, which is no longer ubiquitinated and degraded [47]. In this model, the destruction complex is saturated with phosphorylated  $\beta$ -catenin, and newly formed  $\beta$ -catenin freely translocates to the nucleus. In the absence of a Wnt stimulation,  $\beta$ -catenin is phosphorylated by CK1 and GSK3 $\beta$ , phosphorylated  $\beta$ -catenin is ubiquitinated by  $\beta$ -TrCP in the destruction complex, and ubiquitinated  $\beta$ -catenin is degraded by the proteasome. The destruction complex is recycled for another round of  $\beta$ -catenin degradation (○ Fig. 3). Therefore, the enhancement of GSK3 $\beta$  activity in this model is not a critical step in the inhibition of Wnt signaling as shown in GSK3 $\alpha$ /GSK3 $\beta$  homozygous knock-in ES cells [46,47]. Although excess GCs suppress bone formation, at least in part, through the activation of GSK3 $\beta$ , the pathological importance of the effects of excess GCs in Wnt signaling through Akt requires further clarification.

A Dex treatment was previously shown to increase the expression of Dkk1 and PPAR $\gamma$ 2, reduce the phosphorylation of GSK3 $\beta$  and the amount of nuclear  $\beta$ -catenin, and induce adipocyte differentiation in the clonal mesenchymal progenitor cell line, ROB-C26 [48]. Furthermore, a Dex treatment increases the expression of C/EBP $\alpha$  by inhibiting the DNA hypermethylation of the promoter and induces adipocyte differentiation in bone marrow stromal cells, while the addition of LiCl, which is an inhibitor of GSK3 $\beta$ , alleviates the effects of Dex on the upregulation of C/EBP $\alpha$ , promoter methylation, and the induction of adipocyte differentiation [49] (○ Fig. 2). These findings indicate that

Dex induces adipocyte differentiation through the inhibition of Wnt signaling.

### MicroRNAs Involved in Impaired Osteoblastogenesis by Excess GCs

Several miRNAs, the expression of which is regulated by excess GCs, have been reported (○ Fig. 2). Excess GCs reduce the expression of miR-29a, and a treatment with miR-29a attenuates the adverse effects of GCs on bone by inducing the protein levels of Wnt3a, phosphorylated ERK,  $\beta$ -catenin, and Akt, and the mRNA levels of Runx2 and IGF-1, and by reducing the mRNA expression of Dkk1 and Rankl [50]. Furthermore, miR-29a has been shown to attenuate GC-mediated  $\beta$ -catenin deacetylation and ubiquitination, increase H3K9 acetylation on its own promoter, and enhance its own transcription by reducing the expression of HDAC4 [51]. GCs upregulate the expression of miR-34a-5p during bone marrow stromal cell proliferation, and miR-34a-5p reduces cell cycle-related proteins, including CDK4, CDK6, and Cyclin D1, leading to the inhibition of proliferation. In contrast, GCs reduce the expression of miR-34a-5p during osteogenic differentiation, leading to Notch signaling activation and the inhibition of osteogenic differentiation in bone marrow stromal cells [52]. Dex has been shown to upregulate the expression of miR-199a-5p, which inhibits osteoblast proliferation by reducing FZD4 and Wnt2, and the deletion of miR-199a-5p attenuates Dex-inhibited osteoblast proliferation [53].

### Other Mechanisms Responsible for Impaired Bone Formation by GCs

GCs fail to reduce bone mass or bone formation in GR conditional knockout mice using *Runx2* Cre transgenic mice. However, GC treatments have been shown to reduce bone mass and bone formation in mice carrying a mutation, which only disrupts GR dimerization, at similar levels to those in wild-type mice. These reductions are, in part, due to the inhibited expression of IL-11 and LIF by excess GCs in a manner that is independent of GR dimerization [11] (○ Fig. 2). Dex reduces the expression of Connexin 43 through the Akt-mTOR pathway, and the overexpression of Connexin 43 attenuates Dex-inhibited cell viability and proliferation in MC3T3-E1 cells [54] (○ Fig. 2). A previous study reported that GR interacts with Runx2 and inhibits the expression of Runx2 target genes [55]. Bone restitution during remodeling was recently found to require the recruitment of osteoprogenitors onto the reverse surface of the canopies, which are defined as a continuous layer of elongated cells lining the bone marrow and separated from the bone matrix by osteoclasts, reversal cells, or osteoblasts. A histomorphometric analysis of iliac crest biopsies from patients treated long-term with GCs revealed an increase in reversal surfaces, which lacked any neighboring osteoclasts or osteoids, and the coverage of the canopy [56]. Therefore, a decrease in the canopy is one of the characteristics of GC-induced osteoporosis.

## Osteoclastogenesis Induced by Excess GCs

GCs enhance bone resorption in humans and mice [2,57,58]. Furthermore, a GC treatment increases osteoclastogenesis and bone resorption in vitro [59–61]. Rankl, which is expressed in stromal cells and osteoblast lineage cells, interacts with the cognate receptor Rank, which is expressed in osteoclast precursors and osteoclasts, resulting in osteoclastogenesis and the activation of osteoclasts. Osteoprotegerin (OPG), which is also expressed in stromal cells and osteoblast lineage cells, is a decoy receptor of Rankl and prevents the interaction of Rankl with Rank, thereby inhibiting osteoclastogenesis and osteoclast activation [62]. Enhanced bone resorption with a GC treatment has been explained by excess GCs severely reducing OPG mRNA expression and mildly increasing Rankl mRNA expression in stromal cells and osteoblastic cells, resulting in a marked increase in the RANKL/OPG ratio [60,63–65] (◻ Fig. 2). Further, the reduction in OPG by excess GCs is abolished in *Sost*<sup>-/-</sup> mice, indicating that *Sost* plays an important role in the regulation of OPG expression [37]. A GC treatment was previously found to increase the expression of Rankl, at least in part, through the suppression of miR-17/20a, which targets Rankl [66]. Although osteocytes strongly express OPG under physiological conditions, GC-induced osteocyte apoptosis further reduces OPG secretion to the bone surface and enhances osteoclastogenesis and osteoclast activation [67,68] (◻ Fig. 2). Osteocyte apoptosis itself also enhances osteoclastogenesis, osteoclast activation, and bone resorption [68].

Excess GCs decrease the number of osteoclast progenitors, but increase the number of osteoclasts by prolonging their life span [69]. Furthermore, excess GCs decrease osteoclast numbers in *Hsd11b2* transgenic mice under the control of the *Trap* promoter [14]. Therefore, excess GCs enhance bone resorption by extending the life span of osteoclasts. The autophagy of osteoclasts is also involved in GC-induced bone resorption because the pharmacological or genetic inactivation of autophagy ameliorates GC-induced bone loss by inhibiting osteoclastogenesis [70]. ROS are upregulated by excess GCs, and *N*-acetylcysteine (NAC), a ROS scavenger, has been shown to abrogate the effects of GCs on autophagy and osteoclastogenesis [61]. Therefore, ROS are involved in autophagy and GC-induced osteoclastogenesis (◻ Fig. 2).

A previous study reported that a GC treatment suppresses the bone-degrading capacity of osteoclasts by disturbing the organization of the cytoskeleton through the inhibition of the M-CSF activation of RhoA, Rac1, and Vav3 in wild-type osteoclasts, but not in osteoclast-specific GR-deficient osteoclasts in vitro [15] (◻ Fig. 2). Osteoclast-specific GR-deficient mice are protected from GC-suppressed bone formation, suggesting that GCs suppress bone formation via osteoclasts [15]. The overexpression of calpain 6 (*Capn6*), which is suppressed by Dex and involved in microtubule stability and acetylation as well as  $\beta$ -integrin expression, rescues the GC-mediated disruption of the osteoclast cytoskeleton [71] (◻ Fig. 2). As GC treatment in osteoclastogenesis in vitro increases or suppresses osteoclastogenesis and bone resorption under similar culture conditions, this discrepancy needs to be resolved. It has also been reported that GCs change the osteoclastic resorption mode. In the presence of excess GCs, osteoclasts elongate the excavations they initiated rather than migrating to a new resorption site, inducing deep resorption pits while reducing time-dependent increases in the resorption pit number [72].

## Conclusions

The GC pathway exerts positive effects on osteoblasts and bone formation under physiological conditions. However, excess GCs suppress bone formation by inducing apoptosis, inhibiting the Wnt signaling pathway, and modulating microRNA expression in osteoblasts and osteocytes. Excess GCs enhance bone resorption by suppressing OPG expression and prolonging the life span of osteoclasts (◻ Fig. 2). Although many studies have focused on the role of the Wnt signaling pathway in the pathogenesis of impaired bone formation in excess GCs, the data obtained remain controversial. Furthermore, there are still a number of phenotypic differences in GC treatments between in vitro and in vivo experiments, among cells, among species, and between cancellous bone and cortical bone. Although many possible mechanisms for GC-induced osteoporosis have been reported, their pathological significance still needs to be evaluated.

## Acknowledgements

This work was supported by the grant from the Japanese Ministry of Education, Culture, Sports, Science and Technology to TK (Grant number: 26221310).

## Conflict of Interest

The author declares no conflict of interest.

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