



# Mechanisms of bone anabolism regulated by statins

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## Synopsis

Osteoporosis is a common disease in the elderly population. The progress of this disease results in the reduction of bone mass and can increase the incidence of fractures. Drugs presently used clinically can block the aggravation of this disease. However, these drugs cannot increase the bone mass and may result in certain side effects. Statins, also known as HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase inhibitors, have been widely prescribed for CVD (cardiovascular disease) for decades. Nonetheless, several studies have demonstrated that statins exert bone anabolic effect and may be helpful for the treatment of osteoporosis. Several experiments have analysed the mechanisms of bone anabolism regulated by statins. In the present paper, we review the mechanisms of promoting osteogenesis, suppressing osteoblast apoptosis and inhibiting osteoclastogenesis.

**Key words:** apoptosis, mesenchymal stem cell (MSC), osteoclastogenesis, osteogenesis, osteoporosis, statins

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## INTRODUCTION

As the population ages, senile diseases are becoming epidemic. Older people suffer numerous difficulties from these diseases and osteoporosis is a typical case. Osteoporosis is also a major social problem due to increasing financial burden. According to the 2003–2006 survey by the National Ministry of Health in China [1], 69.4 million people over the age of 50 years in China suffer from osteoporosis (15.3 million men and 54.1 million women) and 213.9 million people in that age group have low bone mass (100.4 million men and 113.5 million women). China spends 10.38 billion yuans annually treating hip fractures resulting from osteoporosis, and this cost is expected to increase in the future. Multiple drugs have been designed to prevent or cure osteoporosis. SERMs (selective oestrogen receptor modulators), various bisphosphonates (oral, injection and intravenous drip), calcitonin and strontium ranelate have been used to treat osteoporosis. However, few of these treatments have

satisfactory effects, and some even lead to further complications [2–5]. Therefore a new effective therapy for osteoporosis is necessary.

Statins, which are usually prescribed to treat and prevent CVD (cardiovascular disease) (Table 1), may be potentially promising drugs for treatment of osteoporosis. Mundy et al. [6] found that statins promote new bone formation in the calvarial bone of neonatal mice, and several studies have confirmed the discovery [7–10]. Sugiyama et al. [11] reported that both compactin and simvastatin increase the expression of BMP-2 (bone morphogenetic protein-2) mRNA and protein in HOS (human osteosarcoma) cells. Osteogenic effects of statins have also been found in other cell lines [7,12–14]. Meanwhile, animal tests have confirmed the osteogenic effects of statins [10,15,16]. Recently, Chuengsamarn et al. [17] reported that statins promote bone formation and increase BMD (bone mineral density) in patients with hyperlipidaemia. Other diseases, such as bone non-union or femoral head necrosis may benefit from statins [18,19]. In addition, statins decrease bone resorption by inhibiting osteoclast differentiation

**Abbreviations used:** ALP, alkaline phosphatase; BMD, bone mineral density; BMP-2, bone morphogenetic protein-2; BMSC, bone-marrow-derived mesenchymal stem cell; CVD, cardiovascular disease; DGBP, digeranyl bisphosphonate; DN, dominant negative; ER, oestrogen receptor; ERK, extracellular-signal-regulated kinase; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GGPPs, GGPP synthase; GR, glucocorticoid receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MAPK, mitogen-activated protein kinase; MKP-1, MAPK phosphatase-1; NF- $\kappa$ B, nuclear factor  $\kappa$ B; OPG, osteoprotegerin; OVX, ovariectomized; PI3K, phosphoinositide 3-kinase; RANK, receptor activator of NF- $\kappa$ B; RANKL, RANK ligand; TGF $\beta$ , transforming growth factor  $\beta$ ; TRAF, tumour-necrosis-factor-receptor-associated factor; TNFR, tumour necrosis factor receptor; ZGA, zaragozic acid.

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**Table 1 The available forms of statins**

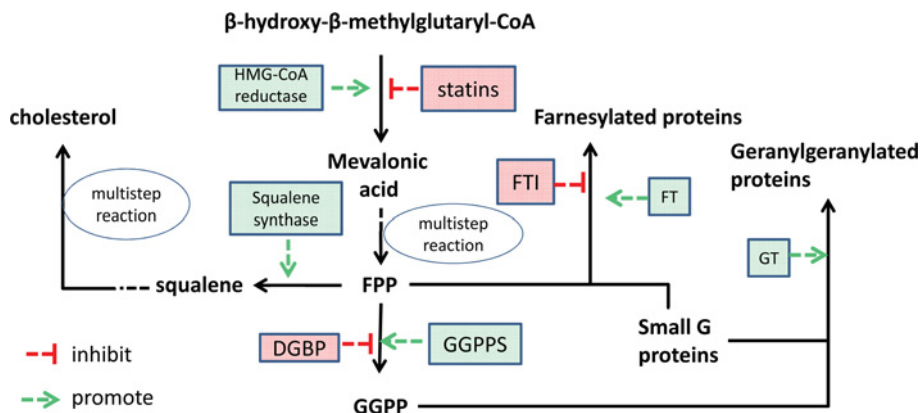
Drug	Molecular formula*	Molecular mass (Da)*	Tissue selectivity	CAS number*	References
Compactin (mevastatin)		390.52	Lipophilic	73573-88-3	[11,31,44,55,60,62]
Lovastatin		404.5	Lipophilic	75330-75-5	[6,9,18,19,21–23,32,34, 80–82]
Simvastatin		418.6	Lipophilic	79902-63-9	[6,7,10–14,16,17,27,33,55,62, 64–67,73,75,76,80–82,86]
Pravastatin sodium		446.52	Hydrophilic	81131-70-6	[11,65,80–85]
Fluvastatin		411.47	Lipophilic	93957-54-1	[15,80–82]
Atorvastatin		1209.42	Lipophilic	134523-00-5	[14,82,85]
Pitavastatin		421.46	Lipophilic	147511-69-1	[8,26,55]
Rosuvastatin		481.54	Hydrophilic	287714-41-4	[39]
Cerivastatin		459.56	Lipophilic	145599-86-6	[27,82]

\*Data from chemBlink (<http://www.chemblink.com>).

and osteoblast apoptosis. Therefore statins may play an important role in orthopaedics owing to their regulatory effects on bone anabolism.

Researchers have attempted to determine the mechanism of bone anabolism regulated by statins. The present review of the

literature suggests that bone anabolism regulated by statins may be attributable to three aspects: promotion of osteogenesis, inhibition of osteoblast apoptosis and suppression of osteoclastogenesis. Some molecules, such as FPP (farnesyl pyrophosphate), GGPP (geranylgeranyl pyrophosphate), Ras and ER (oestrogen



**Figure 1** Biosynthetic route of endogenous cholesterol synthesis

Statins competitively inhibit HMG-CoA reductase and thereby inhibit the synthesis of mevalonic acid. FPP is the downstream mediator of mevalonic acid. Therefore the synthesis of FPP can be blocked by statins. Squalene synthase catalyses FPP to form squalene and then squalene is converted into cholesterol through multiple reactions. FPP can also be covalently added to small G-proteins to produce farnesylated proteins. This process is catalysed by farnesyl transferase (FT) and can be inhibited by an FT inhibitor (FTI). GGPPs converts FPP into GGPP. GGPPs can be inhibited by DGBP. Geranylgeranyl transferase (GT) transfers the geranylgeranyl moiety of GGPP to the small G-proteins to produce geranylgeranyl proteins.

receptor), may play important roles in the bone anabolism regulated by statins.

### Statins promote osteogenesis by inhibiting the synthesis of FPP and GGPP

Osteoblasts, which are essential for bone formation, secrete proteins to form bone matrix and promote bone mineralization. Statins can promote the differentiation of osteoblasts to regulate bone anabolic metabolism, and FPP, GGPP, small G-proteins and BMP-2 are all involved in osteogenesis induced by statins.

### Statins inhibit the synthesis of FPP and GGPP to increase osteogenesis

FPP and GGPP are intermediates in the mevalonate pathway (Figure 1). FPP is converted into squalene by squalene synthase, and then a series of enzymes transforms squalene into cholesterol. FPP may also be bound to small G-proteins or be converted into GGPP by GGPPs (GGPP synthase). This GGPP may then be converted into geranylgeranyl proteins [20]. Statins inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, and thereby block the synthesis of mevalonate. FPP and GGPP are the downstream products of mevalonate. Thus, statins can reduce the synthesis of cellular FPP and GGPP. Tong et al. [21] measured the concentrations of FPP and GGPP in MC3T3 cells, some treated with lovastatin. The concentrations were  $0.125 \pm 0.010$  and  $0.143 \pm 0.008$  pmol/ $10^6$  cells respectively in untreated cells, whereas the concentrations of both decreased in a dose-dependent manner in treated cells.

FPP is one of the components targeted by statins to regulate osteogenesis. Weivoda et al. [22] found that FPP (as well as GGPP) decreased during statin-induced osteoblast differentiation. Another study found that osteoblast differentiation was

inhibited by the expression of exogenous FPP and that increased levels of endogenous FPP were induced by ZGA (zaragozic acid) or DGBP (digeranyl bisphosphonate) [23]. ZGA or DGBP may inhibit the conversion of FPP into squalene or GGPP and thereby increase intracellular FPP, which suggests that statins stimulate osteogenesis, at least partially, by reducing FPP [21,24].

GGPP is also involved in statin-induced osteogenesis. Yoshida et al. [25] reported that GGPP and GGPPs decreased during mineralization in MC3T3-E1 cells. GGPP (geranylgeraniol), which is converted into GGPP by a salvage pathway, inhibits the osteogenic differentiation of MC3T3-E1 cells. This suggests that GGPP may negatively regulate osteogenic differentiation. Ohnaka et al. [26] tested the role of GGPP in pitavastatin-induced osteogenesis and found that incubating primary human osteoblasts with  $1 \mu\text{M}$  pitavastatin for 48 h up-regulated the expression of BMP-2 mRNA; this effect was abolished when the cells were co-cultured with 1 mM mevalonate or  $5 \mu\text{M}$  GGPP. Similar effects were also observed by Maeda et al. [27], who demonstrated that simvastatin stimulated MC3T3-E1 cells to differentiate into osteoblasts. However, the addition of GGPP to the medium containing simvastatin blocked the osteogenic differentiation of MC3T3-E1 cells.

The above studies indicate that statins induce osteogenesis, at least in part, by reducing FPP and GGPP levels. However, how this is related to the promotion of osteogenesis is unclear.

### Small G-proteins prenylated by FPP or GGPP have important effects on osteogenesis

Small G-proteins regulate a series of cellular processes, including gene expression, cytoskeleton formation and vesicular trafficking. The small G-proteins can be classified into more than five families, including the Ras/Rap, Rho/Rap, Rab, Ran and Arf families, are synthesized in the cytoplasm and cannot be activated

until anchored to intracellular membranes [28–30]. Prenylation is a post-translational modification. During prenylation, farnesyl or geranylgeranyl-derived from FPP or GGPP respectively are covalently added to the CAAX tetrapeptide in the C-termini of small G-proteins. Then the small G-proteins are anchored to the intracellular membranes by farnesyl or geranylgeranyl. Thus, FPP and GGPP are crucial for the activation of small G-proteins. Considering that statins inhibit the synthesis of FPP and GGPP, they may also regulate the prenylation of small G-proteins and thereby regulate cellular activities.

Rho, a small G protein, performs many functions in signal transmission. In human endothelial cells, its location on the membrane is affected and its GTP binding activity is inhibited by a high concentration of mevastatin (10  $\mu$ M) [31]. Rho is also involved in statin-induced osteogenesis. Ohnaka et al. [26] found that Rho and its downstream target protein Rho kinase, when activated by LPA (lysophosphatidic acid), played a negative role in bone formation. However, 1  $\mu$ M pitavastatin stimulated the expression of BMP-2 and osteocalcin mRNA by suppressing the activation of Rho and Rho kinase. Therefore the authors suggested that pitavastatin stimulates bone formation by inhibiting Rho and Rho kinase.

Conversely, another small G-protein, Ras, is activated by statins [32,33]. This contradictory effect between Rho and Ras may be explained by the finding that different concentrations of statins may have opposite effects on the activation of small G-proteins; for example, in human umbilical vein endothelial cells, a high concentration of lovastatin (10–50  $\mu$ M) inhibits the prenylation of Ras, Rho and Rap, whereas a therapeutic level (0.05–0.5  $\mu$ M) does not [34]. This therapeutic level could activate Ras and its downstream molecules Akt and ERK (extracellular-signal-regulated kinase) to increase osteogenesis. That study also found that this therapeutic level of lovastatin also significantly decreased cellular cholesterol, and that adding exogenous cholesterol abolished Ras activation. Therefore they inferred that statins stimulate the activity of Ras through the depletion of cholesterol.

### Statins up-regulate BMP-2 by activation of the Ras signalling pathway

BMP-2, a low-molecular-mass glycoprotein, belongs to the TGF $\beta$  (transforming growth factor  $\beta$ ) superfamily. BMP-2 stimulates bone formation by regulating mesenchymal condensation that is important in both intramembranous and endochondral ossification. In addition, it is necessary for the initiation of fracture healing [35] and has already been prescribed to treat non-union and delayed bone union [36,37].

Mundy et al. [6] considered statins a class of previously unknown bone anabolic agents. They linked the BMP-2 promoter to the firefly luciferase reporter gene in an immortalized murine osteoblast cell line and evaluated the effects of more than 30 000 natural compounds (including natural statins) on the BMP-2 promoter by testing the luciferase activity. They found that lovastatin specifically increased luciferase activity, and that fluvastatin, simvastatin and mevastatin had similar effects. Other studies have

confirmed that statins stimulate bone formation by promoting the expression of BMP-2 mRNA in bone cells [7,8,11,14,27,39].

To examine whether the expression of BMP-2 is essential for osteogenesis induced by statins, Ghosh-Choudhury et al. [32] treated 2T3 cells with lovastatin and the BMP-2 antagonist noggin. Treatment with noggin inhibited the expression of osteoblast-specific markers induced by lovastatin and confirmed that BMP-2 is an important molecule for osteogenesis induced by lovastatin. They determined that lovastatin increased the expression of BMP-2 through the Ras/PI3K/Akt/MAPK (mitogen-activated protein kinase)/BMP-2 pathway. Furthermore, using the immune complex kinase assay with anti-phospho-ERK1/2 antibody, they found that both lovastatin and simvastatin activated MAPK. The DN (dominant negative) ERK2 partially inhibited the expression of BMP-2 induced by lovastatin, suggesting that MAPK may regulate lovastatin-induced osteogenesis. Chen et al. [33] confirmed these results and further reported that simvastatin promotes the localization of both RasGRF1 and phospho-RasGRF1 on the intracellular membrane; RasGRF1 accelerates the transformation of Ras protein from the inactive GDP state into the active GTP state; activated Ras promotes the phosphorylation of ERK1/2 through MAPK; Akt is involved in the expression of lovastatin-induced BMP-2; and that lovastatin activates Akt and increases the expression of BMP-2. In addition, they found that DN Akt modestly inhibits the expression of lovastatin-induced BMP-2, and that the activation of Akt and MAPK, and the expression of lovastatin-induced BMP-2 can be blocked by inhibition of PI3K (phosphoinositide 3-kinase). Finally, they reported that the PI3K/Akt pathway for statin-induced osteogenesis is dependent on the activation of Ras, as both inhibitors of Ras, FTI-227 and DN RasN17, inhibit the lovastatin-induced activation of PI3K and Akt.

Franceschi et al. [40] demonstrated that the osteogenic effect induced by BMP-2 is mediated by Runx2, an essential transcription factor for bone formation and osteoblast differentiation [41] that stimulates the transcription of osteoblast-specific genes. Therefore Runx2 may be involved in statin-induced osteogenesis. Statins may increase the expression of Runx2. For example, Li et al. [18] found that statins stimulated bone-marrow MSCs (mesenchymal stem cells) to differentiate into osteoblastic cells by increasing the expression of Runx2. Runx2 responds to many upstream molecules, such as BMP-2 and MAPK [43]. It is known that statins activate MAPK and increase the expression of BMP-2 [32]. However, how Runx2 responds to BMP-2 and MAPK in the bone anabolic effect induced by statins remains unclear.

Experiments performed by Vukelic et al. [44] may also help explain the effects of statins on the expression of BMP-2. They found that FPP activated the GR (glucocorticoid receptor) in skin cells. As activation of GR increases bone resorption and up-regulates the expression of MKP-1 (MAPK phosphatase-1) to block the proliferation of osteoblasts [45,46], the impairment of osteogenesis induced by FPP may be attributable to the activation of GR, and implies that statins may also maintain the activation of the MAPK/BMP-2 signalling pathway by reducing cellular FPP and thereby inhibiting GR activation.

### Statins suppress osteoblast apoptosis through the TGF $\beta$ /Smad3 signalling pathway

The average lifespan of osteoblasts is approximately 3 months. After osteoblasts finally stop secreting bone matrix, 5% become lining cells, 30% become osteocytes and 65% are destined for apoptosis [47,48]. Protection of osteoblasts from apoptosis increases bone formation [49].

Statins may also increase bone formation by inhibiting osteoblast apoptosis through the TGF $\beta$ /Smad3 signalling pathway. The TGF superfamily contains more than 40 proteins involved in tissue homeostasis and determination of cell fate by modulating the cellular process from membrane to nucleus [50]. Centrella et al. [51] found that TGF $\beta$  played a critical role in bone formation. Smad proteins are key components of the TGF $\beta$  signalling pathway [52]. Smad3 is regulated by the TGF $\beta$  receptor. TGF $\beta$  activates type II receptors resulting in the activation of type I receptors, and the phosphorylation of TGF $\beta$  type I receptor-like kinase activates Smad3, which is essential for bone mass maintenance [53]. The deletion of Smad3 results in the decrease of bone formation by stimulating the apoptosis of osteoblasts, conversion of osteoblasts into osteocytes and then the apoptosis of osteocytes. Overexpression of Smad3 in mouse osteoblasts stimulates bone formation by increasing ALP (alkaline phosphatase) activity, mineralization and the synthesis of matrix proteins [54].

Recently, Kaji et al. [55] found that pitavastatin, mevastatin and simvastatin induced the expression of Smad3 in MC3T3-E1 cells and UM-106 cells, suggesting that these statins may antagonize dexamethasone-induced apoptosis in a dose-dependent manner. However, this anti-apoptosis effect was abolished by dominant negative Smad3. Overexpression of Smad3 inhibited osteoblast apoptosis and the use of statins increased the level of Smad3 in osteoblasts. In addition, specific inhibition of TGF $\beta$  by SB431542 rescinded the inhibition of osteoblast apoptosis induced by pitavastatin. Therefore statins may inhibit osteoblast apoptosis through the TGF $\beta$ /Smad3 pathway. The TGF $\beta$ /Smad3 pathway may be independent of BMPs because SB431542 has no effect on BMP signalling [56]. Furthermore, osteoblast apoptosis impairs the balance between bone formation and resorption [57]. The anti-apoptosis effect may partially explain another anabolic effect of statins.

### Statins inhibit osteoclastogenesis by regulating ER and the OPG (osteoprotegerin)/RANKL [RANK (receptor activator of NF- $\kappa$ B) ligand]/RANK signalling pathways

Once formed, bone is in a constant state of remodelling which includes formation and resorption. The majority of bone diseases can be attributed to excessive resorption [58]. Inhibition of osteoclast activity contributes to bone mass preservation. Reportedly, statins can block osteoclastogenesis. Luckman et al. [59] found that compactin induced the apoptosis of osteoclasts in a dose-dependent manner. Woo et al. [60] reported that compactin inhibited the fusion of preosteoclasts and disrupted the actin ring of osteoclasts. The ER plays an important role in the inhibition of osteoclastogenesis; for example, oestrogens inhibit osteoclasto-

genesis by reducing RANKL [61]. ER expression is regulated by statins, and the OPG/RANKL/RANK system is involved in the inhibition of osteoclastogenesis induced by statins [62].

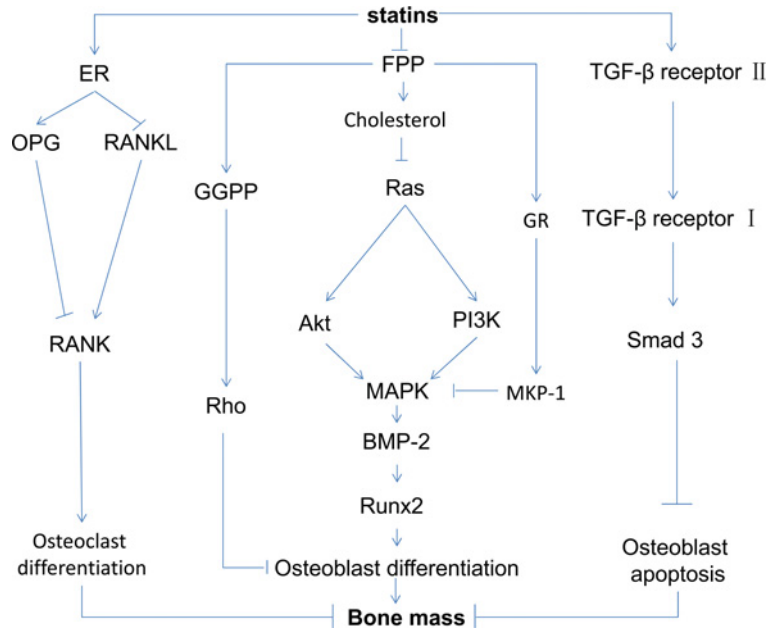
### Statins inhibit osteoclastogenesis by increasing the expression of ER

Oestrogens and their receptors play an important role in bone metabolism [63]. Oestrogens reduce bone resorption. Women suffer from a rapid decrease in bone mass during the first 5–10 years after menopause due to a sharp decrease in oestrogen. ERT (oestrogen replacement therapy) can be used to treat postmenopausal osteoporosis. Animal tests have proven that statins can accelerate fracture healing in OVX (ovariectomized) rats compared with a control group [64]. Several clinical tests have also shown that statins are therapeutic for postmenopausal osteoporosis [65]. Statins are presumably correlated with ER in bone. Song et al. [66] explored the relationship between statins and ER. They treated mouse BMSCs (bone-marrow-derived mesenchymal stem cells) with non-immune normal mouse IgG or BMP-2 neutralizing antibodies together with different concentrations of simvastatin, and found that ALP activity was not completely blocked by neutralizing the BMP-2 monoclonal antibody, while the ER $\alpha$  protein level increased after the mouse BMSCs were treated with simvastatin for 72 h in a concentration-dependent manner. The authors concluded that simvastatin preserves bone by inducing not only BMP-2 but also ER $\alpha$ ; a follow-up study confirmed this conclusion [67]. In it, OVX Sprague–Dawley rats were treated with simvastatin alone, 17- $\beta$ -oestradiol (E2) alone, or a combination of both for 6 weeks, and then the uterine wet weight, BMD of the lumbar vertebrae, biomechanics of the lumbar vertebrae and expression of ER $\alpha$  in the bone and uterus were analysed. The results showed that simvastatin inhibited bone loss induced by ovariectomy and stimulated ER $\alpha$  expression in bone.

The above-mentioned research offers new insight into the mechanisms of statins in bone anabolism. Simvastatin reduces FPP, which is a transcriptional activator of ER. Therefore the role of FPP in the expression of ER $\alpha$  induced by simvastatin may need to be examined.

### Statins decrease osteoclastogenesis by regulating the OPG/RANKL/RANK pathway

The OPG/RANKL/RANK signal pathway is the final mediator in the regulation of osteoclastogenesis [68]. OPG is a soluble glycoprotein that belongs to the TNFR (tumour necrosis factor receptor) superfamily [69]. RANK is a type I transmembrane protein that also belongs to this superfamily and associates with TRAF1 (tumour-necrosis-factor-receptor-associated factor 1), TRAF2, TRAF3, TRAF5 and TRAF6 to activate NF- $\kappa$ B (nuclear factor  $\kappa$ B) and JNK (c-Jun N-terminal kinase) [70]; it also plays an important role in the proliferation and differentiation of osteoclasts. RANKL is a polypeptide that is commonly expressed on the surface of osteoblasts [71]; it stimulates osteoclast activity and blocks osteoclast apoptosis. OPG/RANKL/RANK signalling is mediated by cytoplasmic factors. The cytoplasmic domain of RANKL binds to the TNFR-associated cytoplasmic



**Figure 2 The signal pathway for bone anabolic effect induced by statins**

Statins activate Ras by inhibiting the synthesis of FPP and then stimulate the expression of BMP-2 through the PI3K/Akt/MAPK pathway. BMP-2 up-regulates the expression of the Runx2 gene, and phosphorylated Runx2 stimulates a series of bone-specific gene transcriptions and promotes the differentiation of osteoblasts. The inhibition FPP synthesis inactivates Rho and MKP-1 by decreasing GR production, which abolishes the negative effects of Rho and MKP-1 on osteogenesis. FPP is a transcriptional activator of GR that activates MKP-1 to dephosphorylate MAPKs, and GGPP converted from FPP inhibits the differentiation of osteoblasts by activating Rho. Statins inhibit osteoblast apoptosis by stimulating Smad3. In addition, statins regulate the OPG/RANKL/RANK signalling pathway by increasing the expression of ER, thereby inhibiting osteoclastogenesis.

factors or TRAFs, and then a series of reactions are activated to control the fate of osteoclasts.

Statins modulate osteoclastogenesis through the OPG/RANKL/RANK signal pathway. Kaji et al. [62] examined the expression levels of OPG and RANKL mRNA using semi-quantitative RT-PCR (reverse transcription-PCR) in mouse bone cell cultures. They found that statins increased OPG mRNA expression and decreased RANKL mRNA expression. Ahn et al. [73] used the murine monocytic RAW 264.7 cell system to examine the effect of simvastatin on osteoclasts. By TRAP (tartrate-resistant acid phosphatase) staining, they found that RANKL-induced differentiation of osteoclasts was effectively blocked when cells were treated with simvastatin in an earlier stage and at a higher concentration. Simvastatin inhibited the RANKL-induced activation of NF- $\kappa$ B by inhibiting the phosphorylation and degradation of I $\kappa$ B $\alpha$  (inhibitory  $\kappa$ B $\alpha$ ), which contributed to the activation of NF- $\kappa$ B; the activation of NF- $\kappa$ B is important for the formation of osteoclasts [74]. Ayukawa et al. [75] treated artificial bone defects in rats with simvastatin for 3 consecutive days. At 5 days after simvastatin injection, the expression of RANKL was depressed and there were fewer osteoclasts, while the new forming bone area was larger compared with the control group. Han et al. [76] found similar results, where simvastatin inhibited the activity of osteoclasts and stimulated new bone formation in

rat periodontal tissues. They attributed this result to an increase in local OPG and a decrease in local RANKL.

How statins regulate the OPG/RANKL/RANK signal pathway is unclear. Previous reports have demonstrated that oestrogen can increase OPG expression [77,78] and suppress RANKL expression through an ER-dependent mechanism [79]. Therefore statins may inhibit osteoclastogenesis by regulating ER through the OPG/RANKL/RANK pathway.

## SUMMARY

The regulatory signals discussed above are shown in Figure 2. Statins increase osteogenesis by inhibiting the synthesis of FPP, decreasing cellular cholesterol and activating the Ras-PI3K-Akt/MAPK signalling pathway, thereby increasing the expression of BMP-2 and Runx2. The inhibition of FPP synthesis results in the inactivation of Rho and MKP-1 by decreasing GR production, which can abolish their negative effects on osteogenesis. Osteoblast apoptosis is suppressed through the TGF $\beta$ /Smad 3 pathway activated by statins, and osteoclastogenesis is blocked by ER through the OPG/RANKL/RANK system. Thus, the

effects of statins on bone anabolism include the promotion of osteoblast differentiation, the suppression of osteoblast apoptosis, and the blocking of osteoclastogenesis. However, the signalling pathways involved in the regulation of bone remodelling by statins are varied and complex. There may be other pathways that have not been discovered or cross-talk between pathways that have already been identified. Therefore further studies are needed to illustrate the exact mechanism of bone anabolism regulated by statins.

In addition, although the effects of statins on bone anabolism have been demonstrated in laboratory studies and the mechanisms of these effects are being revealed, they have not been confirmed clinically [80–86]. This is attributable to the low bioavailability of statins in bone, as all of the current statins used clinically target the liver. Therefore if the bioavailability of statins in bone can be improved, further studies in a clinical setting should be conducted to evaluate their therapeutic use.

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