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Role of fibroblast growth factor 2 and Wnt signaling in anabolic effects of parathyroid hormone on bone formation

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Abstract

Osteoporosis poses enormous health and economic burden worldwide. One of the very few anabolic agents for osteoporosis is parathyroid hormone (PTH). Although great progress has been made since the FDA approved PTH in 2002, the detailed mechanisms of the bone anabolic effects of intermittent PTH treatment is still not well understood. PTH bone anabolic effect is regulated by extracellular factors. Maximal bone anabolic effect of PTH requires fibroblast growth factor 2 (FGF2) signaling, which might be mediated by transcription factor activating transcription factor 4 (ATF4). Maximal bone anabolic effect of PTH also requires Wnt signaling. Particularly, Wnt antagonists such as sclerostin, dickkopf 1 (DKK1) and secreted frizzled related protein 1 (sFRP1) are promising targets to increase bone formation. Interestingly, FGF2 signaling modulates Wnt/β-Catenin signaling pathway in bone. Therefore, multiple signaling pathways utilized by PTH are cross talking and working together to promote bone formation. Extensive studies on the mechanisms of action of PTH will help to identify new pathways that regulate bone formation, to improve available agents to stimulate bone formation, and to identify potential new anabolic agents for osteoporosis.

Keywords

PTH; FGF2; Wnt signaling; ATF4

Introduction

Osteoporotic fracture is estimated to occur every 3 seconds worldwide (Johnell and Kanis, 2006). An estimated 44 million Americans are threatened by osteoporosis today. By 2025, more than 3 million osteoporotic fractures are estimated to occur in the United States alone and the cost is predicted to be approximately $25.3 billion (NOF.). Therefore, osteoporosis is an enormous health and economic problem. Osteoporosis is a disease characterized by low bone mass and deterioration in the microarchitecture of bone tissue (Holroyd et al., 2008).

Current treatments for osteoporosis include antiresorptive agents and osteoanabolic agents. Some antiresorptive agents are biophosphonates, estrogen, raloxifene and calcitonin (Vokes and Favus, 2010). The anti-resorptive agents reduce the fracture risk primarily by blocking bone resorption. Human parathyroid hormone (PTH 1-34, Teriparatide) is the only available anabolic agent in clinical use for the treatment of osteoporosis currently in the United States and available throughout most of the world. Intact human PTH 1-84 has been approved in many European countries (Silva and Bilezikian, 2011). Intermittent administration of PTH

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peptide 1-34 or full-length protein PTH 1-84 increases bone formation in osteoporotic patients (Hodsman et al., 2005). The present article will focus on extracellular factors that mediate PTH bone anabolic effects.

Bone Biology

Bones can be thought of as an organ of the skeletal system. They provide mechanical support to soft tissues, and support muscle action and haematopoiesis. They also contribute to mineral homeostasis. These functions are accomplished by continuous bone remodeling which is coupled by bone resorption and bone formation (Harada and Rodan, 2003; Raisz, 2005). Bone resorption is carried out by osteoclasts, which are derived from hematopoietic lineage. Bone formation is carried out by osteoblasts, which are mesenchyme-derived. The bone resorption period is usually rapid within several days while the bone formation period is relatively slow over several months. The complete remodeling cycle takes about 3–6 months (Baron, 2003).

Osteoblasts differentiation

Osteoblasts originate from mesenchymal stem cells. Differentiation of mesenchymal stem cells towards osteoblasts is governed by a series of transcription factors, including runt-related transcription factor 2 (Runx2), osterix and activating transcription factor 4 (ATF4) (Franceschi et al., 2007; Karsenty, 2008), Figure 1. Mature osteoblasts will lay down bone matrix proteins including type I collagen and osteocalcin, which will slowly mineralize. Therefore, type I collagen is considered as an early stage of osteoblasts marker. Mature osteoblasts secrete osteocalcin, which is considered to be a late stage of osteoblasts marker. As cells differentiate, while the majority of osteoblasts die, some osteoblasts become osteocytes embedding in bone matrix and some become bone-lining cells (Baron, 2003).

Osteoclast differentiation

Osteoclasts originate from hematopoietic stem cells and osteoclasts differentiation is regulated by osteoblasts. The factors coupling osteoblasts to osteoclasts include macrophage colony-stimulating factor (M-CSF), the ligand for receptor activator of nuclear factor kappa B (RANKL) and osteoprotegerin (OPG) (Baron, 2003). Bone marrow stromal cells and osteoblasts express M-CSF as well as RANKL. M-CSF stimulates proliferation and differentiation of osteoclast progenitors. RANKL binding to receptor RANK on osteoclast progenitor surface is required for osteoclast differentiation. In addition to RANKL, osteoblasts also secrete OPG, which is a decoy receptor for RANKL and thus a negative regulator for osteoclast differentiation. Therefore, osteoclast maturation is regulated by osteoblasts (Baron, 2003).

Bone-remodeling process is well controlled by coordination of osteoblasts, osteoclasts, osteocytes, bone lining cells and other cells. Unbalanced bone remodeling, such as increased bone resorption and/or inadequate bone formation will results in bone loss. Inadequate bone formation might be due to alternations of commitment, proliferation and differentiation of osteoblasts progenitors, as well as the lifespan of the mature osteoblasts.

Intermittent administration of PTH increases bone formation

The principal form of biological active parathyroid hormone is the intact molecule, PTH (1-84). The amino-terminal (N) fragment, PTH (1-34) has similar biological activity to the full-length parathyroid hormone, PTH (1-84) (Hock et al., 2002). Intact human PTH 1-84 has been approved for therapeutic use in Canada and Europe but not in the United States. Currently teriparatide human PTH (1-34) is the only available anabolic agent in clinical use for the treatment of osteoporosis in the United States and most countries throughout the

world. Intermittent administration of PTH peptide 1-34 or full-length protein PTH 1-84 increases bone formation in osteoporotic patients (Hodsman et al., 2005).

In osteoblast, PTH signals through receptor PTH1R and PTH can stimulate extracellular calcium influx through cAMP-dependent and cAMP-independent pathway or PKC signaling pathway (Hock et al., 2002; Swarthout et al., 2002). Although no PTH receptors were found on osteoclasts, PTH can regulate osteoclast indirectly through osteoblasts. Continuous PTH treatment has been demonstrated to increase RANKL expression and down regulate expression of OPG, thereby favoring osteoclastogeneis and bone resorption (Hock et al., 2002). However, intermittent administration of PTH in vivo has been well documented to increase bone formation (Hock et al., 2002).

PTH intermittent administration stimulates bone formation as well as bone resorption. However, there is a period when PTH enhances bone formation to a greater extent than PTH stimulates bone resorption (Canalis et al., 2007). The period is considered as “the anabolic window”. Considering that the bone anabolic effects of PTH wane after the anabolic window and that long-term effects of PTH administration has not been evaluated, the recommended duration of PTH therapy is relatively short: 2 years in the United States and 18 months in Europe (Canalis et al., 2007). During the anabolic window, it is known that bone formation markers increase before bone resorption markers. Other morphometric observations including bone volume, bone microstructure and mechanical properties also confirmed the anabolic effects of PTH on bone (Canalis et al., 2007).

Intermittent PTH treatment has been demonstrated to increase bone formation by acting on different phases of bone remodeling (Figure 2): stimulating precursors commitment into osteoblasts lineage, promoting osteoblast differentiation, inhibiting osteoblast apoptosis and inhibiting proliferation of osteoblast progenitors (Jilka, 2007). Intermittent PTH treatment increased osteoblasts number also by attenuating progenitors differentiation into adipocytes and by reactivating bone lining cells (Jilka, 2007). In addition, PTH stimulates bone resorption, which release factors important for bone formation (Jilka, 2007). Besides bone cells, bone marrow microenvironment has been recently shown to impact anabolic actions of PTH (Koh et al., 2011). Since the FDA approved intermittent PTH administration in 2002, great progress has been made in understanding how intermittent PTH treatment mediates its bone anabolic effects. However, the detailed mechanisms are still not fully defined.

Our studies suggest that PTH increasing of osteoblasts number and promoting osteoblasts differentiation is partially mediated by extracellular factors, including fibroblast growth factor 2 (FGF2) and Wnt proteins (Figure 3).

Anabolic actions of PTH on bone require FGF2

FGF2 is one of the earliest members identified in the FGF polypeptide family (Hurley et al., 2002). It is expressed in osteoblasts and stored in extracellular matrix (Hurley et al., 2002). The clinical importance of FGF2 in bone has been revealed in recent studies. We observed that FGF2 expression decreased in osteoblasts from aged patients compared to young patients (unpublished data). However, PTH intermittent treatment increased serum FGF2 in osteoporotic patients (Hurley et al., 2005). In addition, FGF2 stimulates proliferation of mesenchymal-derived progenitor cells from aging mouse and human bone (Ou et al., 2010). Furthermore, FGF2 has been used in clinical trials to stimulate periodontal regeneration (Kitamura et al., 2011) and to accelerate healing of tibial shaft fractures (Kawaguchi et al., 2010). Additionally, polymorphisms of the FGF2 gene may contribute to the susceptibility of osteoporosis (Lei et al., 2011). These studies indicate the clinical relevance of FGF2 research.

FGF2 signals through FGF receptors that have tyrosine kinase activity (Powers et al., 2000). Binding of FGF2 to FGF receptors induces receptor dimerization and activation, resulting in activation of downstream signaling. FGF2 downstream signaling pathways include mitogen-activated-protein kinase (MAPK) pathway, the PI-3 kinase-AKT pathway and the phospholipase C (PLC) pathway (Dailey et al., 2005). MAPK pathways with extracellular signal-regulated kinases (Erks), P38 MAP kinases and protein kinase C (PKC) are well studied in osteoblasts (Marie, 2003).

Although sustained FGF2 treatment maintains the osteoblast precursor proliferative state thereby reducing osteoblasts differentiation and inhibiting bone formation (Canalis et al., 1988; Hurley et al., 1993; Rodan et al., 1989), osteoblast precursors are able to differentiate in the absence of continuous FGF2 exposure. Intermittent FGF2 treatment increases osteoblasts differentiation and bone formation (Mayahara et al., 1993; Nakamura et al., 1998). The increased bone formation induced by intermittent FGF2 may also be partially due to enhanced osteoblasts replication (Globus et al., 1988; Ou et al., 2010). This is reminiscent of the actions of PTH where continuous treatment inhibits osteoblast differentiation and new bone formation, whereas intermittent PTH increases bone formation.

The importance of FGF2 in bone is supported by our studies using FGF2 null mice. Disruption of the *Fgf2* gene results in significantly decreased bone mass and bone formation revealed by histomorphometry and micro-CT data (Montero et al., 2000). Furthermore, FGF2 knockout mice display greatly reduced trabecular plate-like structures and loss of connecting rods (Montero et al., 2000). This decreased bone formation phenotype might not be due to reduced progenitors as both wild type and FGF2 knockout BMSCs have similar colony forming efficiency (Xiao et al., 2010). Instead, this decreased bone formation phenotype may be due to alteration of progenitor cell lineage commitment, since FGF2 deficiency results in increased bone marrow adipogenesis and reduced osteogenesis (Xiao et al., 2010). The reduced bone formation may be also due to a defect in osteoblast differentiation as shown by decreased alkaline phosphatase (ALP) positive colonies and von Kossa staining in cultured $Fgf2^{-/-}$ BMSCs (Montero et al., 2000). The decreased colony area can be partially rescued by exogenous FGF2 administration to $Fgf2^{-/-}$ BMSCs *in vitro* (Montero et al., 2000; Naganawa, et al., 2006). FGF2 could also stimulate bone formation *in vivo* (Mayahara et al., 1993; Nakamura et al., 1998). These studies show that FGF2 is a positive regulator of osteoblast differentiation and bone formation.

In addition to the function in osteoblasts differentiation and bone formation, FGF2 is also required for osteoclast formation and bone resorption since FGF2 deletion results in reduced osteoclast formation and resorption both *in vitro* (Okada et al., 2003) and *in vivo* (Montero et al., 2000). FGF2 also negatively regulate adipogenesis as FGF2 deletion results in increased adipogeneis in bone marrow but exogenous FGF2 treatment block adipogensis in BMSCs (Xiao et al., 2010). Therefore, similar to PTH, FGF2 stimulates bone formation also by regulating function of osteoclasts and adipocytes.

Interestingly, other factors important for bone homeostasis also regulate FGF2 expression in osteoblasts, for example: prostaglandins (Sabbieti etal., 1999), transforming growth factor β (Hurley et al., 1994), bone morphogenetic protein-2 (Naganawa et al., 2008), 17β-estradiol (Hurley et al., 1996) and PTH (Hurley et al., 1999; Hurley et al., 2005) all increase FGF2 mRNA and protein.

We previously demonstrated that PTH increases FGF2 and FGF receptor mRNA expression in cultured osteoblasts (Hurley et al., 1999). The mechanisms by which PTH regulates FGF2 involve protein kinase A (PKA)-cAMP and/or PKC pathway activation, since activators for PKA and PKC pathway all increased FGF2 expression, which mimicked the stimulatory

effect of PTH on FGF2 expression (Hurley MM, 1999). Ongoing studies are further characterizing how PTH regulates FGF2. Besides increasing FGF2 in mouse cells, PTH treatment increases serum FGF2 and increases bone formation markers in osteoporotic patients (Hurley et al., 2005). The increased bone formation markers including osteocalcin (OCN) may be partially due to increased FGF2 because FGF2 was shown to induce OCN mRNA expression in cultured osteoblasts (Xiao et al., 2002). FGF2 stimulation of new bone formation *in vivo* is also well documented (Mayahara et al., 1993). However, disruption of FGF2 gene in mice results in dramatic reduction in bone formation and OCN expression (Naganawa, et al., 2006). Moreover, in *Fgf2*$^{-/-}$ mice, the anabolic action of PTH in bone is greatly impaired. PTH treatment significantly increased bone formation in FGF2 wild type mice but the increase was blunted in *Fgf2*$^{-/-}$ mice (Hurley et al., 2006). PTH promoted bone formation by increasing expression of osteoblasts differentiation transcription factor Runx2 and of proteins involved in osteoblasts proliferation and viability, but the increase was greatly attenuated by FGF2 deficiency (Sabbieti et al., 2009). *Therefore, anabolic effects of PTH on bone formation require endogenous FGF2*. However, the downstream target of FGF2 signaling is not well understood.

The impaired anabolic effect of PTH in *Fgf2*$^{-/-}$ mice is in part due to reduced ATF4 expression

Recent studies identify transcription factor ATF4 as a novel downstream target gene of PTH signaling in osteoblasts (Yu et al., 2008). ATF4 is a transcription factor important for osteoblasts terminal differentiation and mineralization (Yang et al., 2004). PTH induces ATF4 mRNA/protein expression in a time and dose dependent manner. In addition, PTH increases ATF4 transcriptional activity in MC-4 cells and in BMSCs (Yu et al., 2008). PTH increases expression of OCN mRNA and protein in a time and dose dependent manner in cultured osteoblasts (Jiang et al., 2004; Yu et al., 2008). However, PTH could not induce OCN expression in ATF4 knockdown MC-4 cells or in *Atf4*$^{-/-}$ BMSCs (Yu, et al., 2008). Moreover, PTH stimulated bone formation was significantly reduced or lost in *Atf4*$^{-/-}$ mice (Yu et al., 2009). These results indicate that *the transcription factor ATF4 mediates the anabolic actions of PTH in bone*.

Expression of transcription factor ATF4 is regulated by FGF2 in osteoblasts, as shown in our recent paper (Fei et al., 2010). We studied the ability of FGF2 to increase expression of ATF4 in BMSCs and examined ATF4 expression in FGF2 deficient BMSCs. We observed that FGF2 increased ATF4 mRNA expression as early as 20min and stimulated ATF4 protein expression after 3 hours of treatment in BMSCs. In addition, ATF4 expression was markedly reduced in cultured BMSCs from *Fgf2*$^{-/-}$ mice compared to wild type mice. We further showed that exogenous FGF2 increased ATF4 expression in *Fgf2*$^{-/-}$ BMSCs as well as in *Fgf2*$^{+/+}$ BMSCs. These results suggest that impaired bone mass and bone formation in *Fgf2* null mice may be due in part to reduced ATF4 expression and that anabolic bone action of FGF2 is partially mediated by ATF4.

Further, we showed that the impaired anabolic effect of PTH in *Fgf2*$^{-/-}$ mice is in part due to reduced ATF4 expression both *in vitro* and *in vivo* (Fei et al., 2011b). *In vitro* data showed that PTH treatment increased ATF4 mRNA expression as early as 15 min in *Fgf2*$^{+/+}$ BMSCs, but no significant increase was observed in *Fgf2*$^{-/-}$ BMSCs. *In vivo* data showed that there was a 13.8% increase in lumbar vertebrae bone mineral density in *Fgf2*$^{+/+}$ mice after 2 weeks intermittent treatment with PTH (1-34) (40ug/kg/d). In contrast, there was a 2.1% decrease in FGF2 deficient mice after PTH intermittent treatment. Consistent with increased bone formation, PTH treatment also enhanced expression of genes that are important for osteoblasts differentiation including type I collegen, osteocalcin and Runx2. However, the increase was attenuated in FGF2 deficient mice. Interestingly, basal

expression of ATF4 mRNA in tibiae was significantly lower in *Fgf2* null mice compared to wild type mice. PTH treatment significantly increased ATF4 mRNA in *Fgf2* wild type mice, but there was no significant increase in *Fgf2* null mice. In addition, ATF4 protein expression measured by immunohistochemistry on lumbar vertebrae tissue was increased by PTH treatment in $Fgf2^{+/+}$ mice but the increase was attenuated in $Fgf2^{-/-}$ mice. The attenuated ATF4 expression might reduce osteoblasts differentiation, and possibly contributes to the impaired anabolic action of PTH on bone formation in $Fgf2^{-/-}$ mice. One needs to note that the impaired PTH bone response in FGF2 deficient mice on a new genetic background of black swiss/129/FVB/N (Fei et al., 2011b), although with lower dosage of PTH, is consistent with the data reported in mice on a black swiss/129Sv genetic background (Hurley et al., 2006). These data further support that *maximal anabolic actions of PTH in bone require FGF2 signaling, which might be mediated by transcription factor ATF4.*

Building bone by PTH/Wnt signaling

Wnt signaling pathway also mediates anabolic action of PTH on bone. Wnt signaling is particularly important for bone homeostasis. Wnt can either signal through non-canonical pathway or the canonical Wnt/β-catenin pathway. The non-canonical β-catenin independent pathway includes Wnt/Calcium pathway and Wnt/planar cell polarity pathway (Piters et al., 2008). The canonical Wnt/β-catenin pathway is well studied in osteoblasts. In the absence of Wnt ligand, the destruction complex including kinase GSK3β will degrade the key signaling factor β-catenin. In order to initiate the signaling cascade, Wnt ligand binds to receptor LRP5/LRP6 and Frizzled. This binding will block the destruction complex, therefore, β-catenin will be stabilized and accumulate in the nucleus. Nucleus β-catenin binds to transcription factor Lymphoid enhancer-binding factor 1/T cell specific transcription factor (LEF/TCF) and activate expression of downstream target genes such as Runx2 to promote osteoblasts differentiation (Piters et al., 2008). The importance of Wnt signaling pathway is well demonstrated in human mutations. Patients with loss of function mutations in the receptors LRP5 (Gong et al., 2001) or LRP6 (Mani et al., 2007) are characterized with low bone mineral density and skeletal fragility. In contrast, patients with gain of function mutations in LRP5 (reduced affinity of LRP5 for Dkk1) have high bone mass (Ai et al., 2005; Boyden et al., 2002; Van Wesenbeeck et al., 2003).

Wnt/β-Catenin signaling regulating osteoblasts differentiation and bone formation involves multiple mechanisms. Wnt/β-Catenin signaling stimulates progenitors commitment into osteoblasts lineage and attenuates progenitors differentiation into adipocytes. Wnt/β-Catenin signaling also promotes osteoblasts proliferation, differentiation and mineralization. In addition, Wnt/β-Catenin signaling represses osteoclasts differentiation by increasing the ratio of OPG/RANKL (Krishnan et al., 2006). Thus, Wnt/β-Catenin signaling stimulation of bone formation is through multiple mechanisms, which is common in the anabolic effects of PTH as well as of FGF2 in bone.

The function of Wnt/β-Catenin signaling on bone formation is negatively regulated by Wnt antagonists including sclerostin (Kamiya et al., 2008), dickkopfs (Dkks) (Kawano and Kypta, 2003), and secreted frizzled related proteins (sFRPs) (Bodine et al., 2004; Yao et al., 2010). Sclerostin and DKKs block Wnt/β-Catenin signaling by binding to one subunit of the Wnt receptor complex. In contrast, sFRPs bind to Wnt proteins and inactivate all Wnt pathway (Kawano and Kypta, 2003). Dkk1 has been demonstrated to antagonize canonical Wnt signaling and inversely correlates with bone mass (Li et al., 2006; MacDonald et al., 2007; Mao et al., 2001; Morvan et al., 2006). Sclerostin (Brunkow et al., 2001; Li et al., 2008; Li et al., 2009), sFRP1 (Bodine et al., 2004; Yao et al., 2010) and sFRP4 (Cho et al., 2010; Nakanishi et al., 2008; Nakanishi et al., 2006) also negatively regulate bone mass.

Wnt Signaling pathway also mediates the anabolic action of PTH on bone. Micro-array analysis revealed that both *in vitro* and *in vivo* PTH treatment regulates genes of the Wnt signaling family (Kulkarni et al., 2005; Li et al., 2007; Onyia et al., 2005; Qin et al., 2003). PTH treatment increased the key factor of Wnt signaling β-catenin in a dose and time dependent manner (Tobimatsu et al., 2006 ; Wan et al., 2008). In addition, PTH treatment significantly increased nuclear localized β-catenin protein in a fracture repair model (Kakar et al., 2007). However, knocking down β-catenin using siRNA blunted the increase of genes important for osteoblast differentiation Runx2, OCN, ALP by PTH treatment (Tian et al., 2011). The mechanisms by which PTH stimulation of β-catenin involves PKA and PKC pathways since activators of PKA and PKC pathway all increased β-catenin expression, however, the specific inhibitors of PKA and PKC pathway antagonized PTH induction of β-catenin levels (Tobimatsu et al., 2006). PTH stimulation of β-catenin levels was also mediated by Smad3 (Inoue et al., 2009; Tobimatsu et al., 2006). Another mechanism by which PTH facilitates β-catenin signaling is through inactivation of kinase GSK-3β (Suzuki et al., 2008). These data support that the Wnt signaling key factor pathway β-catenin mediates bone anabolic effect of PTH.

Furthermore, recent data show that PTH receptor PTH1R interacts with Wnt co-receptor Lrp6, thereby activating Wnt/β-catenin signaling (Wan et al., 2008). PTH1R can also regulate Wnt/β-catenin signaling independent of Lrp6. Another Wnt co-receptor is frizzled and frizzled requires adaptor protein dishevelled to activate Wnt/β-catenin signaling. It has been shown that PTH1R can directly interact with disheveled, independent of Lrp6, to regulate β-catenin signaling (Romero et al., 2010).

PTH activation of Wnt/β-catenin signaling not only stimulates osteoblasts differentiation and bone formation (Tobimatsu et al., 2006; Wan et al., 2008) but also induces osteoclastogenesis (Romero et al., 2010).

Besides canonical Wnt/β-catenin signaling, PTH has also been demonstrated to activate non-canonical Wnt signaling in bone. PTH treatment stimulates expression of non-canonical Wnt ligand Wnt 4 in osteoblasts and this stimulation is primarily through PKA pathway (Bergenstock and Partridge, 2007; Li et al., 2007).

In addition to direct regulation of Wnt signaling in bone by PTH, PTH also regulates Wnt signaling indirectly through Wnt antagonists. As described above, Wnt antagonist sclerostin negatively regulates Wnt signaling pathway. *Sost* is the gene that encodes protein sclerostin and sclerostin is mainly expressed by osteocytes (van Bezooijen et al., 2004; Winkler et al., 2003). Sclerostin has been reported to bind to receptor LRP5/6 (Li et al., 2005; ten Dijke et al., 2008), thereby antagonizing β-Catenin signaling and negatively regulating bone formation. Patients with sclerostin deficiency in sclerosteosis or Van Buchem disease display tremendous increase in bone mass and BMD throughout the skeleton (Van Hul et al., 1998). Similarly, mice deficient of *Sost* exhibit a high bone mass phenotype (Kramer et al., 2010; Li, et al., 2008), in contrast, mice overexpressing sclerostin display a low bone mass phenotype (Kramer et al., 2010; Loots et al., 2005; Winkler et al., 2003). These studies confirmed the negative regulation of sclerostin in bone. Recently, sclerostin was shown to be involved in the anabolic bone effect of PTH. Patient studies showed that serum sclerostin levels negatively correlate with PTH levels (Ardawi et al., 2011; Mirza et al., 2010) and intermittent PTH therapy in patients reduced circulating sclerostin levels (Drake et al., 2010). Animal studies confirm that sclerostin is a target for PTH in bone. PTH treatment induced a dramatic reduction of Sost mRNA and sclerostin *in vitro* as well as *in vivo* (Bellido et al., 2005; Keller and Kneissel, 2005). Furthermore, intermittent PTH treatment induced bone gain is blunted in *SOST* overexpression and deficient mice due to attenuated

bone formation (Kramer et al., 2010). These data indicates that PTH anabolic effects involve suppression of sclerostin, an inhibitor of bone formation.

The studies discussed above suggest that sclerostin might be a promising target to induce bone formation. Indeed, using sclerostin antibody to enhance bone formation has been under intense study. Blocking the endogenous inhibitor sclerostin using antibody will lead to activation of canonical Wnt signaling, which will stimulate bone formation. Sclerostin antibody treatment has been reported to enhance bone formation in aged osteoporotic animals (Agholme et al., 2010; Li, et al., 2009) as well as in young animals Agholme et al., 2010). In addition, sclerostin antibody treatment enhances bone formation not only in loss of bone (osteoporotic) setting (Li, et al., 2009), it also enhances fracture healing and strengthens bone density and strength of non-fractured bones. Ominsky et al., 2011). Importantly, increased bone formation and bone mass induced by sclerostin antibody is not affected by pretreatment or co-treatment with anti-resorptive drugs (Li et al., 2011). All these data indicate that sclerostin antibody is a promising agent for the treatment of osteoporosis or to enhance fracture healing in patients.

Notably, PTH is still able to increase bone formation in the absence of sclerostin Kramer et al., 2010), supporting that other factors such as FGF2 (Fei et al., 2011b; Hurley et al., 2006), ATF4 (Yu et al., 2009) and other Wnt signaling regulators also play important roles in PTH bone anabolic effects.

Another Wnt antagonist DKK1 negatively regulates Wnt/β-Catenin signaling by binding and antagonizing receptor LRP5/6 (He et al., 2004). DKK1 has been demonstrated to be a down-regulator of bone formation: deletion of a single allele of the *DKK1* gene in mice results in increased bone formation (Morvan et al., 2006); in contrast, overexpression of DKK1 inhibits Wnt/β-Catenin signaling in bone and leads to osteopenia (Li et al., 2006). In addition, serum DKK1 levels was significantly increased in postmenopausal osteoporotic patients (Anastasilakis et al., 2010). Moreover, DKK1 neutralization antibody has been reported to protect from systemic bone loss during inflammation (Heiland et al., 2010) and induce bone formation in young growing animals as well as in traumatic injury models (Li et al., 2011). These data suggest that DKK1 negatively regulates bone formation and DKK1 antibody is a promising agent to increase bone formation in certain conditions. The role of DKK1 antagonizing Wnt/β-Catenin signaling is also important in the bone effect of PTH. Suppression of Wnt signaling by Dkk1 attenuates PTH-mediated stromal cell response and new bone formation (Guo et al., 2010). Interestingly, targeted overexpression of Dkk1 in osteoblasts reduces bone mass but does not impair the anabolic response to intermittent PTH treatment in mice (Yao, et al., 2011). Therefore, further studies are needed to examine the role of DKK1 in the bone effect of PTH.

In addition, Wnt antagonist sFRP1 is also involved in PTH bone anabolic effects. sFRP1 is one member of sFRP family and sFRPs antagonize Wnt signaling by binding to Wnt ligands and preventing Wnt/receptor activation (Bovolenta et al., 2008). SFRP1 has been shown to be a negative regulator for bone formation: sFRP1 overexpression inhibits bone formation (Yao et al., 2010) and sFRP1 deficiency enhanced trabecular bone formation in mice (Bodine et al., 2004). Interestingly, decreased sFRP1 expression was associated with increased bone formation in low molecular mass FGF2 transgenic mice; in contrast, increased sFRP1 expression was associated with reduced bone formation in low molecular mass FGF2 knock out mice (Xiao et al., 2009). These data indicate that sFRP1 is a target of FGF2 signaling and future studies will determine the detailed mechanism by which FGF2 regulates sFRP1. Additionally, sFRP1 inhibitor has been reported to stimulate bone formation (Bodine et al., 2009). Consistent with the negative bone formation regulatory role of sFRP1, sFRP1 overexpression attenuates PTH bone anabolic effects. On the other hand,

bone anabolic effects of PTH are blunted in the absence of sFRP1 (Bodine et al., 2007). These data suggest that maximal PTH anabolic for bone requires a certain amount of sFRP1.

Summary and future directions

Overall, this article summarizes the role of extracellular signals of FGF2 and Wnt signaling in PTH bone anabolic effects. As one of the very few potent anabolic agents for osteoporosis, PTH increases osteoblasts number, stimulates osteoblasts differentiation and bone formation through multiple ways (Figure 2). This PTH bone anabolic effect is mediated by extracellular factors. Maximal bone anabolic effect of PTH requires FGF2 signaling, which might be mediated by transcription factor ATF4. Maximal bone anabolic effect of PTH also requires Wnt signaling. Particularly, Wnt antagonists such as sclerostin, DKK1 and sFRP1 are promising targets to increase bone formation. Interestingly, we recently demonstrated that FGF2 stimulation of bone formation is modulated by Wnt/β-Catenin signaling (Fei et al., 2011a) (Figure 3). Therefore, the multiple signaling pathways utilized by PTH are cross talking and working together to promote bone formation. Extensive studies on the mechanisms of action of PTH will help to identify new pathways that regulate bone formation, to improve available agents to stimulate bone formation, and to identify potential new anabolic agents for osteoporosis.

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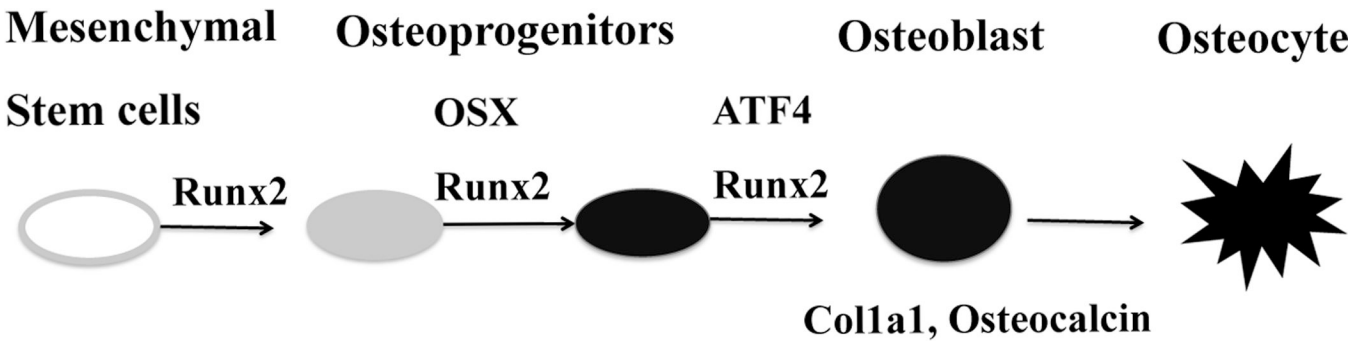

Figure 1.
Osteoblast differentiation from mesenchymal stem cell to mature osteoblast is regulated by transcriptions factors. (Adapted from (Franceschi, et al., 2007))

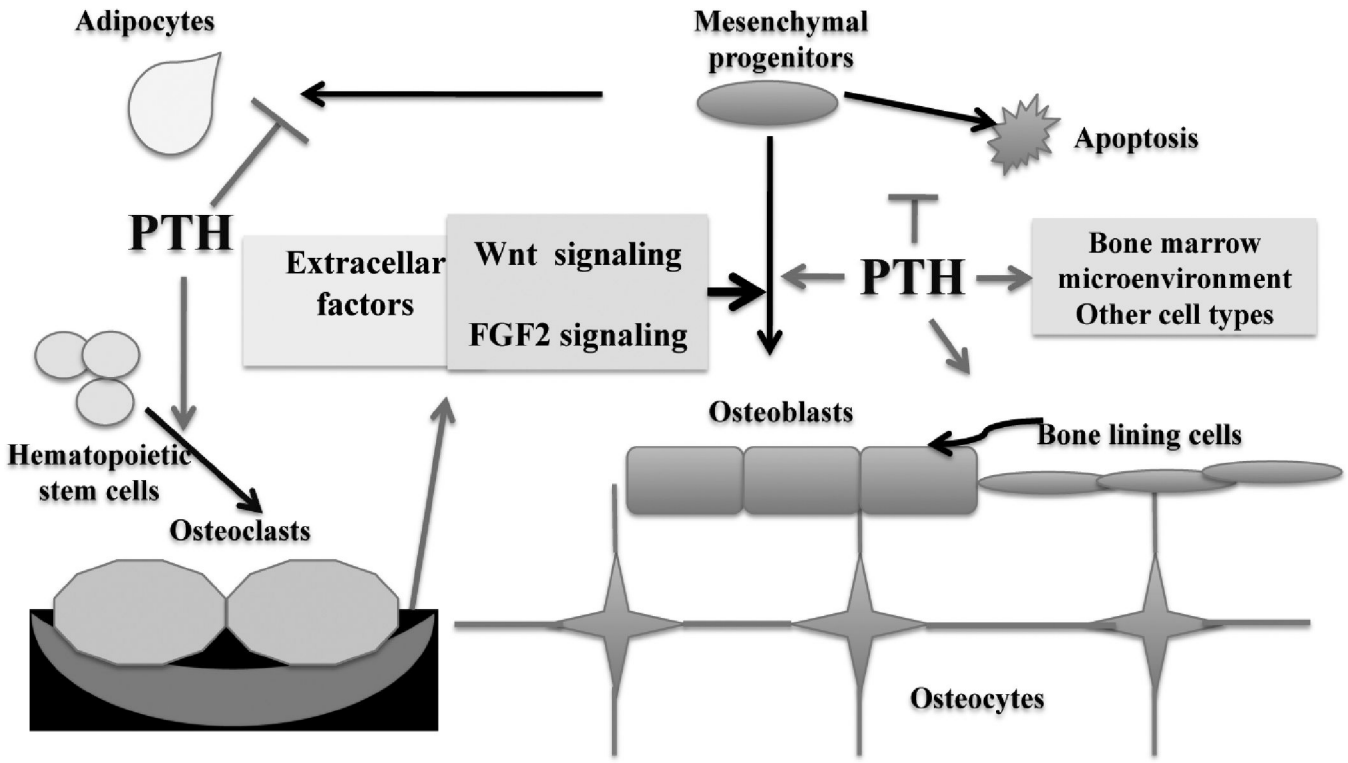

Figure 2.
Intermittent PTH administration stimulates osteoblast differentiation and bone formation through multiple mechanisms (Adapted from (Jilka, 2007)).

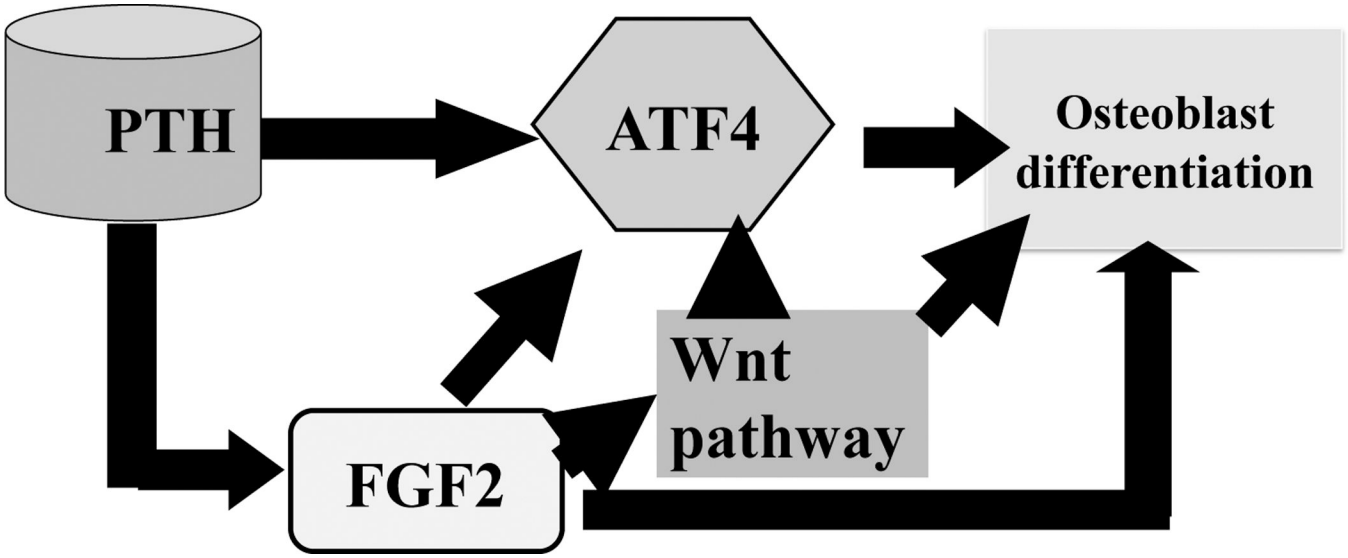

Figure 3.
Bone anabolic effect of PTH is mediated by signaling of extracellular factors, such as FGF2 and Wnt signaling.