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Insulin Like Growth Factor-I: A Critical Mediator of the Skeletal Response to Parathyroid Hormone

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Abstract

This review focuses on the mechanisms by which PTH stimulates both osteoblast and osteoclast function, emphasizing the critical role that IGF-I plays in these processes. After reviewing the current literature on the skeletal actions of PTH and the modulation of IGF action on bone by the different IGF-binding proteins, the review then examines studies from mouse models in which IGF-I or its receptor have been selectively deleted in different cells of the skeletal system, in particular osteoprogenitors, mature osteoblasts, and osteoclasts. Mice in which IGF-I production has been deleted from all cells are deficient in both bone formation and bone resorption with few osteoblasts or osteoclasts in bone *in vivo*, reduced osteoblast colony forming units, and an inability of either the osteoblasts or osteoclast precursors to support osteoclastogenesis *in vitro*. Mice in which the IGF-I receptor is specifically deleted in mature osteoblasts have a mineralization defect *in vivo*, and bone marrow stromal cells from these mice fail to mineralize *in vitro*. Mice in which the IGF-I receptor is deleted in osteoprogenitor cells have a marked reduction in osteoblast proliferation and differentiation leading to osteopenia. Finally mice lacking the IGF-I receptor in their osteoclasts have increased bone and decreased osteoclast formation. PTH fails to stimulate bone formation in the mice lacking IGF-I or its receptor in osteoblasts or enhance the signaling between osteoblasts and osteoclasts through RANKL/RANK and Ephrin B2/Eph B4, emphasizing the role IGF-I signaling plays in cell-communication per se and as stimulated by PTH.

Keywords

Insulin like growth factor; osteoblasts; osteoclasts; osteoprogenitors; parathyroid hormone

PARATHYROID HORMONE/IGF INTERACTIONS: AN OVERVIEW AND INTRODUCTION TO A WORKING MODEL

Parathyroid hormone (PTH) stimulates both bone formation and resorption, the balance of which is dependent on the pharmacodynamics of administration. When given intermittently PTH is anabolic for most skeletal sites [1–3]. This has led to approval of PTH for the treatment of osteoporosis. Continuous exposure to PTH is catabolic as its stimulation of bone resorption exceeds that of bone formation [4, 5]. PTH is thought to stimulate bone resorption primarily by induction of receptor activator of NF-kB ligand (RANKL) and

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suppression of osteoprotogerin (OPG) by osteoblasts resulting in the differentiation and subsequent activation of osteoclast precursors [4]. These effects *in vivo* can be reproduced *in vitro*. The mature osteoblast appears to be an important target for these actions of PTH in that the receptor for PTH (PTH-R) increases with differentiation (maturation) of the osteoblast, and the ability of PTH to induce RANKL and osteoclastogenesis parallels PTH-R expression [6]. Thus the effects of PTH on osteoclastogenesis appear to be mediated by its action on osteoblasts.

The mechanism by which PTH stimulates bone formation remains less well understood. PTH administration *in vivo* increases osteoblast number *in vivo* and osteoprogenitor number and their subsequent differentiation *in vitro* [7, 8]. One possibility is that early osteoprogenitors are directly stimulated by PTH to proliferate and differentiate. We have recently shown that PTH *in vitro* can acutely (within minutes) increase markers of proliferation and differentiation, but such changes are transient, and if PTH exposure is prolonged inhibition of proliferation and differentiation results [9]. On the other hand stimulation of osteoprogenitor proliferation and differentiation by PTH may require signals from other cells. As noted above, we have observed that the PTH-R levels increase with osteoblast maturation, suggesting that mature osteoblasts may be the target for PTH, which in turn elaborate paracrine factors such as insulin like growth factor-I (IGF-I) that act on the osteoprogenitors [7]. Furthermore, inhibition of osteoclastogenesis (c-fos null mouse) or osteoclast function (bisphosphonate treatment) blocks the ability of PTH to stimulate bone formation implicating osteoclasts or their precursors in the anabolic actions of PTH [10]. The role of the osteoclast in osteoprogenitor proliferation/differentiation may involve direct cell-cell interactions (*e.g. via* ephrinB2/EphB4 signaling) [11] or the elaboration of paracrine signals such as IGF-I from the osteoclast to the osteoblast [12].

We propose that IGF-I, a growth factor induced by PTH in osteoblasts, is required for the anabolic and catabolic actions of PTH on bone. This proposal comes from our studies with various animal models in which IGF-I and its receptor have been deleted in specific cell types in the skeleton. IGF-I stimulates osteoprogenitor proliferation and differentiation [13] as well as osteoclast formation *in vitro* [14]. Mice in which IGF-I production has been deleted from all cells (IGF-IKO) are deficient in both bone formation and bone resorption with few osteoblasts or osteoclasts in bone *in vivo*, reduced osteoblast colony forming units, and an inability of either the osteoblasts or osteoclast precursors to support osteoclastogenesis *in vitro* [14, 15]. Mice in which the IGF-I receptor is specifically deleted in mature osteoblasts (IGF-IRobKO) have a mineralization defect *in vivo* [16], and bone marrow stromal cells (BMSC) from IGF-IRobKO fail to mineralize *in vitro* [7]. When the IGF-IR is deleted in osteoprogenitors (IGF-IRopKO), a reduction in osteoblast number and proliferation is observed in addition to decreased osteoblast differentiation and mineralization [17]. Mice lacking the IGF-IR in osteoclast precursors (IGF-IRoclKO) have increased bone and decreased osteoclastogenesis [18]. PTH fails to stimulate bone formation in the IGF-IKO or IGF-IRobKO. Of particular interest is the observation that the IGF-IR in the mature osteoblast is required for the ability of PTH *in vivo* to stimulate osteoprogenitor cell proliferation and differentiation measured *in vitro* [7] indicating a clear requirement for signaling from the mature osteoblast to the osteoprogenitor to mediate this action of PTH.

Our working model proposes that PTH stimulates IGF-I production by the osteoblast, and the IGF-I so produced promotes the proliferation and differentiation of osteoprogenitors as well as facilitating the ability of the mature osteoblast to terminally differentiate and promote osteoclastogenesis (Fig. 1). IGF-I plays a paracrine role to stimulate osteoprogenitor proliferation and differentiation. Similarly, IGF-I, RANKL, and m-CSF elaborated by the mature osteoblast under PTH stimulation promote osteoclastogenesis, which in turn facilitates osteoprogenitor proliferation and differentiation perhaps also by

releasing IGF-I and/or *via* the bidirectional signaling of ephrinB2/EphB4. At this stage we favor the mature osteoblast as the major site for PTH regulation of these events but cannot exclude the alternative and not mutually exclusive possibility that PTH has its major impact on osteoprogenitors, and may also directly act on osteoclast precursors. This brief review will provide the evidence for implicating IGF-I in the anabolic and catabolic actions of PTH.

SKELETAL RESPONSE TO PTH

Intermittent administration of PTH has proven a remarkably effective therapy for osteoporosis. It increases bone mineral density at the hip and spine and reduces fractures [1–3]. However, PTH is a two edged sword in that continuous administration increases bone resorption more than formation (continuous PTH administration may actually decrease bone formation in some models) [4, 5] resulting in bone loss. The catabolic actions may also apply in normal physiology in that hypoparathyroid subjects (humans and mice) have increased bone mass relative to normal or hyperparathyroid subjects [19, 20]. Both gender and region of bone examined can influence the results [21–24]. Studies in rodents consistently show a positive anabolic action of intermittent PTH on both cancellous and cortical bone [25], although regional differences are still apparent [26, 27]. The developmental stage of the animal also makes a difference. The PTH null mouse at birth shows decreased cartilage matrix mineralization, decreased neovascular invasion with decreased expression of angiopoietin-1, decreased trabecular bone volume (proximal tibia), and decreased numbers of osteoblasts and osteoclasts [28]. However, these defects resolve postnatally such that by 2mo of age no apparent growth plate abnormalities can be seen, and both trabecular and cortical bone are increased over wildtype controls, although bone formation and osteoclast number are still reduced [20]. In contrast, PTHrP null mice die shortly after birth with gross distortion of the hypertrophic zone and decreased chondrocyte proliferation [29]. We have observed that the global deletion of IGF-I [15] or the chondrocyte specific deletion of IGF-IR [30] resembles the more severe phenotype of the PTHrP null mouse, whereas the deletion of IGF-IR in osteoblasts [7, 16] more closely resembles that of the PTH null mouse, providing initial evidence that lack of IGF-I signaling may impair responsiveness to PTHrP in cartilage and PTH in bone.

The site specific target cells for PTH are not totally defined. Rouleau *et al.* [31] identified a spindly shaped cell in the intertrabecular region of the metaphysis which accounted for the majority of binding to radiolabelled PTH infused into the animal. Subsequent studies including our own using immunolocalization and in situ hybridization have localized the PTH receptor (PTH-R) in chondrocytes and osteoblasts of the primary and secondary spongiosa [13, 32, 33]. Spindle shaped mononuclear cells located between blood vessels and osteoblasts, osteocytes, and flat lining cells have also been shown to have the PTH-R [33, 34]. In cultures of primary mouse osteoblasts, we [6] have observed that the expression of the PTH-R increases as the bone cells differentiate, suggesting that PTH-R is found in highest levels in the mature osteoblast. However, this does not exclude their existence in less differentiated osteoblasts or even stem and myeloid precursor cells.

The mechanism by which PTH stimulates bone formation is unclear. PTH, given intermittently, stimulates bone formation more than bone resorption, an effect associated with a rapid increase in the mRNA levels for runx2, osteocalcin, alkaline phosphatase, and collagen 1 [26, 35]. When bone marrow stromal cells (BMSC) are evaluated *in vitro*, more colony forming units and an increased percentage of alkaline phosphatase positive colonies are observed when the cells are taken from animals treated *in vivo* with PTH [7, 26], suggesting that PTH promotes the proliferation and/or differentiation of osteoblast precursors, in addition to stimulating the function of the mature osteoblast, although increased survival of osteoblasts may also contribute [36]. We and others have shown that

PTH administration to bone cells *in vitro* results in rapid and transient increases and/or activation of transcription factors associated with proliferation and differentiation such as c-fos, c-jun, c-myc, CREB, and runx2 [8, 32, 37, 38], and directly stimulates proliferation and differentiation when administered transiently [9, 39–41]. These data support an effect of PTH on both the osteoprogenitor and the mature osteoblast. Prolonged exposure was inhibitory [6, 9, 13, 41].

Several studies suggest that osteoclasts or osteoclast precursors are required for these anabolic actions of PTH. Clinical studies have demonstrated that bisphosphonates, which inhibit osteoclast function and promote their apoptosis, block much of the increase in BMD following PTH treatment [2, 3]. c-fos null mice (which lack functional osteoclasts) fail to respond to PTH [10]. However, other studies in which osteoclast numbers and/or function were inhibited with either alendronate or osteoprotegerin (OPG) did not show marked suppression of the anabolic actions of PTH [42]. Recently it has been shown that osteoclasts expressing ephrinB2 can stimulate early osteoblast differentiation *via* the ephrinB2 receptor EphB4 in osteoblasts, supporting the concept of a role for osteoclast/osteoblast interactions that could promote osteoblast proliferation and differentiation [11]. Of interest is that this ephrinB2:EphB4 interaction results in a decrease in osteoclastogenesis (unlike RANKL:RANK interaction) providing a potential mechanism by which PTH can increase anabolism over catabolism if it were to promote the ephrinB2:EphB4 interaction. We [12] have shown that PTH stimulates transcription of ephrinB2 and EphB4 in bone and bone marrow stromal cells (BMSC), an action blocked by deletion of the IGF-IR in osteoblasts [17]. Similar results with respect to PTH stimulation of ephrin B2 have recently been reported by Allan *et al.* [43].

PTH rapidly stimulates RANKL *in vivo* and *in vitro* and stimulates the ability of mature osteoblasts to promote osteoclastogenesis [4, 6, 27, 44]. Of particular interest is that PTH acutely increases OPG expression as well, but prolonged exposure (24 hr) inhibits OPG expression but not RANKL expression [4, 6, 44]. Thus the differential effect of short vs prolonged exposure to PTH on osteoblast proliferation and differentiation relative to the promotion of osteoclastogenesis may underlie the differential effect of short vs prolonged exposure to PTH with respect to the balance of its anabolic and catabolic actions. In either case PTH requires the IGF-I signaling pathway to be fully effective.

PTH stimulates a number of signaling pathways in bone cells including PKA and PKC activation, which can lead to activation of transcription factors such as runx2, members of the AP-1 family such as c-fos and c-jun, CREB, and C/EBPδ [38, 45]. However, most studies indicate that PKA activation is sufficient for most if not all of the anabolic actions of PTH [36, 46]. A C/EBPδ response element is found in the untranslated region of the first exon of the IGF-I gene, and provides the mechanism by which PTH (and other activators of PKA) induce IGF-I production. More recently the wnt signaling pathway has been implicated with the demonstration that PTH inhibits Dkk1 [47, 48] and SOST [49] expression (the products of which are inhibitors of wnt signaling acting at the level of LRP5/6) in a manner also linked to PKA activation [47]. However, PTH has been shown to activate wnt signaling in mice overexpressing Dkk1 [48] and retains its anabolic effects on bone in mice null for LRP5 [50, 51] indicating that at least the initial stages of canonical wnt signaling pathway may not be critical for the anabolic actions of PTH. In this regard PTH has recently been shown to stimulate the phosphorylation of and so inactivate GSK3β in a manner blocked by the PKA inhibitor H89 but not by Dkk1, consistent with a downstream regulation of wnt signaling [48].

Although stimulation of the wnt signaling pathway is an attractive model to explain PTH action, our investigations have focused on the IGF-I signaling pathway. The observations

that PTH stimulates IGF-I production by osteoblasts *in vivo* and *in vitro* [45, 52–54], that the stimulation of IGF-I expression coincides with that of runx2 and precedes that of alkaline phosphatase and osteocalcin, that IGF-I directly stimulates osteoblast proliferation and differentiation *in vitro* [13], and that blocking IGF-I action *in vitro* [52] or deleting it [23] or its receptor *in vivo* and *in vitro* blocks the anabolic actions of PTH provide strong evidence that IGF-I is an essential mediator of PTH action.

ROLE OF IGF-I IN MEDIATING THE SKELETAL RESPONSE TO PTH

As previously noted PTH increases the mRNA and protein levels of IGF-I in bone and bone cells both *in vivo* [53, 54] and *in vitro* [52, 55]. IGF-I mRNA and protein are found in highest concentrations in the osteoblasts in the region of bone (primary spongiosa) most responsive to PTH [56]. PTH administration further increases IGF-I mRNA and protein in that region [54]. Continuous PTH has been reported to decrease IGF-I expression compared to intermittent PTH [57], providing one explanation for the observation that continuous PTH has a catabolic rather than anabolic effect on bone. The anabolic action of PTH on bone *in vivo* is blunted in hypophysectomized rats [58] and growth hormone deficient patients [59], but is restored with GH at least in part because of the induction by GH of IGF-I production in bone [60]. IGF-I and its homologous family member IGF-II are both made by bone cells. IGF-I is the dominant IGF in postnatal murine bone, whereas IGF-II dominates in human bone. Although IGF-II has its own receptor, the IGF-IR is the major means by which these growth factors regulate cell growth and differentiation [61].

Liver specific deletion of IGF-I production has minimal impact on skeletal growth [62], but when combined with deletion of the gene for the acid labile subunit to further reduce circulating IGF-I levels, a moderate reduction in bone size and bone mineral density (BMD) is observed [63]. Thus, circulating IGF-I has a role in bone remodeling, but endogenous, *i.e.* skeletal, IGF-I production is likely to play the major role [64, 65].

REGULATION OF IGF ACTION BY IGF-BINDING PROTEINS

Osteoprogenitor cells express all six of the high affinity IGF-binding proteins (IGFBP or BP), and chondrocytes express all but BP-1 [66]. The expression level of these BPs changes with differentiation of the cells [66]. The impact of these BPs on the action of IGF on bone is quite complex, varying according to the BP being evaluated, the molar ratio with IGF, type and differentiation level of cell being evaluated, effect of matrix, posttranscriptional modification, and proteolytic processing [66]. In general all BPs by binding IGF have the potential to inhibit IGF action. However, substantial differences exist among the different BPs. Transgenic overexpression of BP-2 leads to skeletal deficiency [67], but in equimolar concentrations with IGF-II BP-2 can promote rat osteoblast differentiation [68]. This is thought to be due to its binding to the matrix and increasing IGF availability to the osteoblast [69]. The rare syndrome of osteosclerosis in patients with hepatitis C is associated with increased BP-2 [69] perhaps by this mechanism. Transgenic overexpression of BP-3 also leads to a reduction in bone, due to both an increase in osteoclastogenesis and decrease in osteoblast proliferation [70]. However, in combination with equimolar concentrations of IGF-I BP-3/IGF-I increased bone mass in ovariectomized rats [71]. Transgenic overexpression of BP-4 decreased osteoblast number and trabecular bone [72]. However, proteases such as pregnancy associated plasma protein A (PAPP-A) are also expressed in bone and by cleaving BP-4 can release the bound IGF, promoting bone formation [73]. BP-5 is the most abundant BP in both bone and cartilage [66]. Transgenic overexpression of BP-5 results in a gender specific (males more than females) and site specific (periosteal, not endosteal) reduction in bone formation [74, 75], although subcutaneous injection of BP-5 over bone increased trabecular bone formation in ovariectomized mice [76]. This may be

due to the ability of BP-5 to bind to matrix, which decreases its affinity for IGF, and so releasing the IGF in the vicinity of osteoblasts [77]. BPs 1 and 6 have received less study in bone. PTH stimulation of BP-5 expression has been observed [78], but this has not been reported for other BPs.

LESSONS FROM IGF-I TRANSGENIC MICE (TABLE 1)

Mice lacking the ability to produce IGF-I (IGF-IKO) are born small (approx 60% normal weight) and if they survive the birth process fail to grow normally such that by 8 wks of life they are 30% of normal weight [15, 79, 80]. A similar phenotype was found in the one human case reported with an IGF-I deletion [81]. Mice lacking the IGF-IR globally do not survive. Although bone formation is reduced in the IGF-IKO, trabecular bone volume (BV/TV) in the proximal tibia is increased [15], a result reflecting the dual effect of IGF-I on osteoblast and osteoclast activity [14]. Colony forming units are reduced in BMSC cultures from these mice. These mice do not respond to either the anabolic or catabolic actions of PTH [23]. Although the anabolic action of IGF-I is well appreciated, IGF-I, like PTH, is also catabolic. This is indicated by the decrease in BV/TV seen in mice over expressing IGF-I under the control of the collagen IAI promoter [65], although an earlier study in which IGF-I was overexpressed in osteoblasts using an osteocalcin promoter showed an increase in BV/TV at six weeks (but not at 3 or 24 wks) [64]. Furthermore, BV/TV is increased in IGF-IKO [15]. Osteoclasts have IGF-IR [82, 83], may produce IGF-I themselves [56, 84], and IGF-I stimulates RANKL expression and osteoclastogenesis in BMSC [14, 85, 86]. Deleting IGF-IR from osteoclast precursors *in vitro* blocks osteoclastogenesis [14], and in coculture studies these cells block the ability of PTH to stimulate the expression of osteoblast differentiation markers including RANKL. Osteoblasts from IGF-IKO have decreased expression of RANKL and are poor stimulators of osteoclastogenesis [14]. This can be reversed with the addition of IGF-I. Thus, both osteoblasts and osteoclasts are producers and targets of IGF-I, and IGF-I signaling is critical to both the anabolic and catabolic actions of PTH.

To distinguish which cells are responsible for the IGF-IKO phenotype and its lack of responsiveness to PTH, we and others have selectively deleted IGF-I and its receptor from osteoclast precursors and osteoblasts of different maturity. Increased BV/TV and decreased osteoclast numbers were observed when we specifically deleted the IGF-IR from osteoclasts using a TRAP5b driven cre recombinase (IGF-IRoclKO) [18]. When the IGF-IR was selectively deleted from mature osteoblasts using an OCN driven cre recombinase (IGF-IRobKO), bone mineralization was reduced, despite normal numbers of osteoblasts [16]. Although the number of colony forming units in BMSC cultures from such mice was normal, the colonies failed to mineralize [30]. In contrast when we deleted IGF-IR from osteoprogenitors using an osterix driven cre recombinase (IGF-IRopKO), osteoblast numbers, proliferation, and differentiation were all reduced resulting in decreased BV/TV [17]. Both IGF-IRobKO and IGF-IRopKO show blunted responses to PTH [7, 17]. In contrast to our studies in which postnatal survival and growth were well maintained in the osteoblast specific knockouts, Govoni *et al.* [87], using a collagen 1α2 driven cre recombinase to delete IGF-I from osteoblasts, observed high perinatal mortality and poor growth, with little change in BV/TV in cancellous bone. However, this cre recombinase is not specific for bone, but was found in high levels in muscle and other non skeletal tissues [87].

CONCLUSIONS

Our data are consistent with a major effect of IGF-I signaling in regulating osteoblast proliferation, differentiation, and regulation of osteoclastogenesis. The anabolic and

catabolic actions of PTH are dependent on such signaling. Although the IGF-I receptor plays a critical role in IGF-I signaling, the actual mechanisms by which IGF-I exerts its effects on the various cells of the skeleton remain under active investigation. IGF-I most likely acts in both a paracrine and autocrine fashion, and regulates other cell communication modalities such as RANKL/RANK and EphrinB2/EphB4. Our findings do not discount the importance of other signaling pathways such as wnt, but indicate that one cannot understand the anabolic and catabolic actions of PTH without first considering the role of IGF-I.

Acknowledgments

ABBREVIATIONS

AKT	protein kinase B
BFR	bone formation rate
BMD	bone mineral density
BMSC	bone marrow stromal cells
BV/TV	bone volume/tissue volume
c-fms	colony stimulating factor receptor
CREB	cyclic AMP response element binding
C/EBP	ccaat element binding protein
Dkk	dickkoff related protein
GH	growth hormone
GSK3β	glycogen synthase kinase 3 beta
IGF	insulin like growth factor
IGF-IR	insulin like growth factor I receptor
IGF-IRKO	deletion of insulin like growth factor I receptor
IGF-IRobKO	deletion of insulin like growth factor I receptor in osteoblasts
IGF-IRoclKO	deletion of insulin like growth factor I receptor in osteoclasts
IGF-IRopKO	deletion of insulin like growth factor I receptor in osteoprogenitors
IGFBP	insulin like growth factor binding protein
IRS	insulin receptor substrate
LRP5	lipoprotein receptor related protein 5
MAPK	mitogen activated protein kinase
m-CSF	macrophage colony stimulating factor
OB	osteoblast
OCl	osteoclast
OPG	osteoprotegerin
PI3K	phosphatidyl inositol 3 kinase

PKA	protein kinase A
PTH	parathyroid hormone
PTHR	parathyroid hormone receptor
RANK	receptor activator of NFkB
RANKL	receptor activator of NFkB ligand
SOST	the gene for sclerostin

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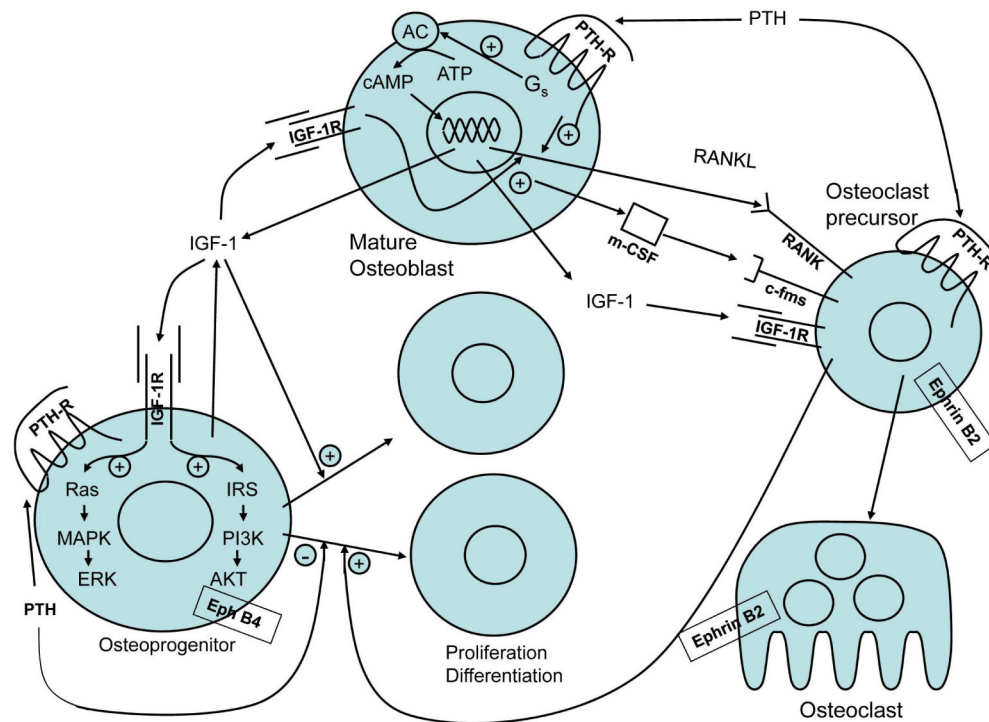
Fig. 1. The role of IGF-I in the anabolic and catabolic actions of PTH: working model
PTH stimulates IGF-I production in osteoblasts through a cAMP dependent mechanism. The
IGF-I so produced enhances the proliferation and differentiation of osteoprogenitors, at least
in part by activating both the MAPK and PI3K pathways, and enables the expression of both
RANKL and m-CSF which along with IGF-I promote the differentiation of osteoclasts. The
osteoclasts in turn facilitate osteoblast differentiation *via* Ephrin B2:EphB4 signaling, the
expression of which is also dependent on IGF-I.

Table 1

Deletion models of IGF and IGF-IR and their Influence on PTH action.

Model	Impact on bone	Effect on PTH actions	reference
Global IGF-IKO	↓ BFR ↑ BV/TV ↓ Ocl	Blocks effect on BFR	[23]
Osteoblast specific IGF-IR KO	↓ Mineralization normal OB	Blocks effect on osteoprogenitor proliferation and differentiation	[7, 16]
Osteoprogenitor specific IGF-IR KO	↓ OB ↓ BV/TV ↓ Mineralization ↓ Differentiation	Blocks stimulation of RANKL and ephrin b2 expression	[17]
Osteoclast specific IGF-IR KO	↓ Ocl ↑ BV/TV	ND	[14, 18]

BFR: bone formation rate; BV/TV = bone volume/total volume; Ocl = osteoclast; OB = osteoblast; ND = not done.