THEMATIC REVIEW

The skeleton: a multi-functional complex organ. New insights into osteoblasts and their role in bone formation: the central role of PI3Kinase

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Abstract

Studies on bone development, formation and turnover have grown exponentially over the last decade in part because of the utility of genetic models. One area that has received considerable attention has been the phosphatidylinositol 3-kinase (PI3K) signaling pathway, which has emerged as a major survival network for osteoblasts. Genetic engineering has enabled investigators to study downstream effectors of PI3K by directly overexpressing activated forms of AKT in

cells of the skeletal lineage or deleting *Pten* that leads to a constitutively active AKT. The results from these studies have provided novel insights into bone development and remodeling, critical processes in the lifelong maintenance of skeletal health. This paper reviews those data in relation to recent advances in osteoblast biology and their potential relevance to chronic disorders of the skeleton and their treatment.

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Introduction

Skeletal tissue in vertebrates develops in two ways, through either intramembranous or endochondral ossification. The former is required for cranial development, while the latter is the mechanism for bone formation in the limb and axial skeleton. Osteoblasts, the bone forming cells of the skeleton, are essential in both these pathways (Kronenberg 2003). Osteoblasts originate from mesenchymal stem cells (MSC) that are multipotent and can differentiate into a number of lineages including bone, fat, muscle, and cartilage. This allocation is dependent on extracellular signaling and the activation or repression of transcription factors that affect common intracellular signaling networks. The Wnt/ β -catenin and the insulin/growth factor/phosphatidylinositol 3-kinase (PI3K) pathways, have been the focus of recent work and both have emerged as critical for bone development, skeletal remodeling and energy metabolism.

The Wnt/ β -catenin pathway has been the subject of several very recent reviews (Baron & Rawadi 2007, Secreto *et al.* 2009, Williams & Insogna 2009) whereas the latter (i.e. insulin/growth factor/PI3K pathway) is often considered

solely within the framework of ligand activation rather than as a final common pathway that targets critical transcription factors to promote MSC growth and differentiation. This review outlines some of the recent advances in our understanding of the PI3K signaling system and its role in bone formation. We will first discuss the osteoblast transcriptional factors that control osteogenesis and then delineate how these are related to PI3K signaling and its downstream targets. Finally, we will discuss potential therapeutic applications of these studies.

PI3K and transcriptional control of osteoblastogenesis

PI3K is an important lipid kinase that controls a number of cellular functions including proliferation, survival, and motility in response to ligand activation. On the contrary, constitutively active PI3K signaling can lead to neoplastic proliferation because of unrestricted cell growth (Cantley, 2002). Therefore, cells have developed a mechanism to regulate this process utilizing phosphatase and tensin homolog deleted on chromosome ten (PTEN; Franke *et al.* 1997, Manning & Cantley 2007). *Pten* was first identified as a tumor suppressor gene and later on was characterized as a lipid phosphatase (Li *et al.* 1997, Maehama & Dixon 1998). PTEN works mainly by acting as a direct antagonist to the actions of

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PI3K, where it can dephosphorylate phosphatidylinositol 3,4,5-trisphosphate (PIP₃) to phosphatidylinositol 4,5-bisphosphate (PIP₂) thereby negatively regulating PI3K signaling as shown in Fig. 1 (Myers *et al.* 1998). In the absence of *Pten* there is accumulation of PIP₃ at the membrane to recruit PDK1 that can phosphorylate AKT at its threonine 308 residue (Alessi *et al.* 1997). This, in turn, leads to mechanistic target of rapamycin complex 2 (MTORC2) phosphorylating AKT at serine 473 (Sarbasov *et al.* 2005), resulting in a completely active AKT pathway.

Recent genetic studies of PI3K signaling reveal that AKT and its downstream targets are critical regulators of endochondral ossification. For example, double *Akt1/Akt2* knockout mice have delayed bone ossification (Peng *et al.* 2003), whereas *Akt1* knockout mice have shorter bones and delayed formation of secondary ossification centers (Ulici *et al.* 2009). AKT has also been shown to affect bone formation and osteoblast survival by regulating Forkhead group of transcriptional factor 3 (FOXO3; Kawamura *et al.*

2007). In addition, AKT in concert with bone morphogenetic protein 2 (BMP2) mediates osteoblast differentiation from mesenchymal stromal cells (Ghosh-Choudhury *et al.* 2002, Mukherjee *et al.* 2010).

FOXOs

In addition to its relatively direct role in bone formation, AKT, an AGC family kinase, can also phosphorylate other families of substrates that are critical for bone formation. Among those is the FOXOs. These transcriptional factors are not osteoblast specific as opposed to other nuclear factors such as Runx2, Osterix, and ATF4. The Forkhead group of transcriptional family members FOXO1, FOXO3, and FOXO4 are highly expressed in chondrocytes and osteoblasts (Ambrogini *et al.* 2010). Activated AKT can phosphorylate FOXOs and control their transcriptional activity by maintaining their retention in the cytoplasm rather than promoting their nuclear transport (Brunet *et al.* 1999).

Figure 1 The model depicted above shows an osteoblast cell where phosphatidylinositol 3-kinase (PI3K) signaling is activated by a growth factor in this case insulin or insulin-like growth factor 1 (IGF1) by binding to their respective tyrosine kinase receptors which leads to binding of substrate proteins like insulin receptor substrate 1 (IRS1) that can bind to PI3K and activate it. PI3K can phosphorylate and convert phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-trisphosphate (PIP₃) that leads to activation of AKT. AKT can phosphorylate and activate MTOR, the downstream effects of this action have not been elucidated in detail during osteogenesis. AKT can also phosphorylate and inactivate Forkhead group of transcriptional factors 1 and 3 (FOXO1 and 3) that leads to regulation of a number of genes that are important during osteoblastogenesis, the figure shows fibroblast growth factor 18 (FGF18) and Runx2 and Sprouty2 (*Spry2*). Osteocalcin (*Bglap2*) that is also among the genes regulated then acts in an endocrine fashion and affects glucose homeostasis.

FOXO1 and FOXO3 have a major role in bone development by negatively regulating the transcriptional expression of key osteoblastic genes such as osteocalcin (*Bglap2*) and *ATF4* (Rached *et al.* 2010a,b). FOXO1, by modulating the expression of osteocalcin and upregulating *Esp* (protein tyrosine phosphatase), also plays a role in glucose homeostasis (Kousteni 2010, Rached *et al.* 2010a,b) and both these studies showed that deletion of FOXO's strongly affects osteoblast function (Table 1). Furthermore, a study when all three FOXO factors were deleted showed an increase in osteoblast and osteocyte apoptosis due to enhanced oxidative stress resulting in loss of bone mass (Ambrogini *et al.* 2010). Most importantly, Ambrogini *et al.* (2010) demonstrated that FOXO3 was the major factor expressed in bone and overexpression in bone led to an increase in bone mass. The role of FOXO4 is not clear in osteoblasts and chondrocytes even though its expression has been shown in these cells.

Runx2

MSCs that enter the osteogenic lineage can be identified by expression of an important transcriptional factor called Runx2. Runx2 is a member of the broader RUNX family of transcriptional factors. In humans, haploinsufficiency of Runx2 causes cleidocranial dysplasia that leads to delays in ossification of the skull and bone formation. Mouse studies of Runx2 deletion reveal embryonic lethality due to the absence of bone formation. Runx2 controls osteoblast differentiation by upregulating the expression of several critical osteoblastic genes including osteopontin (*Spp1*), bone sialoprotein (*Bsp*), and osteocalcin (*Bglap2*) (Ducy *et al.* 1997, Mundlos *et al.*

1997, Otto *et al.* 1997). Runx2 also interacts with PI3K signaling networks at various levels to control expression of p85 and p110 β subunits of the PI3K complex (Fujita *et al.* 2004). Runx2 binds to the promoter regions of these genes and interacts with coactivators thereby inducing gene expression. Recent studies have shown that FOXO1, that can be negatively regulated by active AKT signaling, can bind to RUNX2 and negatively regulate its transcriptional activity specifically relative to osteocalcin gene expression (Yang *et al.* 2011).

Osterix

Another important osteoblastic gene that is a target of Runx2 during osteoblast differentiation is Osterix (*Sp7*). It is a zinc finger-containing protein homologous to the Sp group of transcriptional factors. Gene deletion studies have shown that in the absence of Osterix there is no bone formation even though there is expression of Runx2 suggesting that Osterix is downstream of Runx2 (Nakashima *et al.* 2002). There is also some evidence suggesting that Osterix can be transcriptionally upregulated by Runx2 (Nishio *et al.* 2006). The expression of this transcriptional factor starts around 12.5 dpc and has a major role during embryonic bone development. But deleting Osterix at different time points postnatally showed that it is also necessary for adult bone growth and resorption, by affecting osteoblast and osteocyte development and function (Zhou *et al.* 2010). Osterix has been shown to transcriptionally upregulate *Col1a1* expression through its interaction with NFATc1 (Koga *et al.* 2005). However, there is no direct evidence showing that Osterix gene expression can be regulated through PI3K signaling

Table 1 Summarizes all the different knockouts and promoter Cre lines utilized to delete Pten and its downstream targets in different skeletal lineages along with some of the key observations

Conditional knockouts of Pten generated by using the following mice	References	Skeletal phenotypes observed
1) a) <i>Col2a1cre/Pten</i> flox/flox (exons 4 and 5)	Ford-Hutchinson <i>et al.</i> (2007)	Disrupted normal growth plate organization, increased skeletal formation with increased AKT and MTOR activity
b) <i>Col2a1cre/Pten</i> flox/flox (exons 4 and 5)	Yang <i>et al.</i> (2008)	Caused dyschondroplasia, decreased chondrocyte proliferation and differentiation with increased endoplasmic stress
c) <i>Col2a1cre/Pten</i> flox/flox (exon 5)	Hsieh <i>et al.</i> (2009)	Kyphosis, larger disorganized growth plates and vertebrae, increased marrow adipocytes
2) Osteocalcin cre/ <i>Pten</i> flox/flox (exons 4 and 5)	Liu <i>et al.</i> (2007)	Decreased apoptosis of osteoblasts leading to increased bone formation through the animals life span
3) Dermo1cre/ <i>Pten</i> flox/flox (exons 4 and 5)	Guntur <i>et al.</i> (2011)	Deletion in osteoprogenitors led to increased osteoblast differentiation due to increased FGF signaling
4) <i>Akt1/Akt2</i> knockouts	Peng <i>et al.</i> (2003)	Delayed bone formation
5) <i>Akt1</i>	Kawamura <i>et al.</i> (2007)	Decreased bone formation and osteoclast-mediated resorption
6) myrAKT (under the control of <i>Col2a1</i> promoter)	Rokutanda <i>et al.</i> (2009)	AKT regulates endochondral bone formation (acting on GSK3, MTOR, and FOXO)
7) <i>Col1a1cre/FOXO1</i>	Rached <i>et al.</i> (2010a,b)	Osteoblast-specific knockout led to decreased bone mass
8) a) FOXO1,3,4 and b) FOXO3 overexpression	Ambrogini <i>et al.</i> (2010)	a) Decreased bone formation and b) increased bone formation

although it has recently been suggested that Osterix gene expression can be upregulated by PI3K signaling in concert with BMP2 signaling (Mandal *et al.* 2010). Additional indirect evidence from targeted FOXO1 knockout mice show there is a decrease in Osterix expression implicating PI3K in the regulation of Osterix (Rached *et al.* 2010a,b).

ATF4

Another major transcriptional factor that is osteoblast specific is *Atf4* (CREB2, cyclic AMP response element-binding protein 2) that belongs to the CREB group of transcriptional factors. Deletion of *Atf4* in mice proved that it was essential for terminal osteoblast differentiation (Yang *et al.* 2004). ATF4 is phosphorylated by RSK2 during bone development and accumulates in osteoblasts where it enhances the synthesis of matrix proteins required for bone formation by stimulating amino acid transport into osteoblasts. ATF4 also controls the expression of 1) osteocalcin which in its uncarboxylated form modulates not only bone formation but also glucose metabolism and 2) the expression of *Esp* which codes for a tyrosine phosphatase (OST-PTP) in mouse osteoblasts and acts as a key negative regulator of insulin receptor activity (Yoshizawa *et al.* 2009). Under conditions of increased oxidative stress, FOXO1 has been shown to physically interact with ATF4 in osteoblasts to upregulate protein synthesis (Rached *et al.* 2010a,b). ATF4 is also important for modulating bone resorption by regulating the expression of RANKL, the protein required for osteoclast differentiation (Yoshizawa *et al.* 2009).

A number of other important transcriptional factors have emerged in the last half decade that regulate osteoblast differentiation such as CREB (Pearman *et al.* 1996), β -catenin (Day *et al.* 2005, Holmen *et al.* 2005), and Twist1 and Twist2 (Bialek *et al.* 2004) among others. Published data reveals that PI3K signaling and its downstream effectors like AKT interact mainly with osteoblast transcriptional factors at the level of gene transcription (Fujita *et al.* 2004, Rached *et al.* 2010a,b). Insulin signaling and bone development also are intricately linked, since osteoblasts secrete and deposit osteocalcin, which can act hormonally to enhance insulin secretion and promote insulin sensitivity. Interestingly, the AKT-FOXO axis in osteoblasts also has been shown to regulate glucose homeostasis. Osteoblasts also express tyrosine kinase receptors for insulin and insulin-like growth factors (IGFs) that through ligand binding can activate downstream PI3K related events. This might be one of the major mechanisms through which PI3K signaling activates bone formation (Fig. 1). Not surprisingly, emerging evidence suggests there is also significant synergy between the AKT/PI3K pathway and Wnt activation of β -catenin. Although the interactions between PI3K and other signaling pathways and transcription factors in osteoblasts are critical, it is clear that this system must be tightly regulated and most importantly, context specific.

Pten signaling and endochondral ossification

Through genetic engineering studies a key role in bone development has emerged for *Pten*, the lipid phosphatase that inactivates PI3K as well as for the PI3K/AKT pathway. Three chondrocyte and one osteoblast specific *Pten* conditional knockout studies have been published delineating the role of *Pten* during endochondral ossification. These studies are extremely valuable since they shed light on the context-specific nature of the PI3K signaling pathway in bone and the potential interaction with other signaling pathways.

Chondrocyte-specific deletion of Pten

Ford-Hutchinson *et al.* (2007) used a *Col2a1Cre* to selectively delete *Pten* in mice. The Cre recombinase is under the control of the collagen2a1 promoter and starts expression at 9.5 dpc and its expression in chondrocytes is specific (Ovchinnikov *et al.* 2000). The authors utilized the *Pten* flox/flox mice described by Suzuki *et al.* (2001) with flox sites flanking exons 4 and 5 of *Pten*. Deletion of *Pten* led to increased chondrocyte differentiation and trabecular bone. Histological survey showed that the growth plates were abnormal with disruption in the arrangement of chondrocytes. The increase in trabecular bone was also followed by an increase in marrow adipocytes in 5-week-old animals. Another group (Yang *et al.* 2008) utilizing the same mice to look at chondrocyte-specific deletion of *Pten*, identified that loss of PTEN in the growth plate led to an increase in endoplasmic stress levels which induced HIF1 α activation. They concluded there was impairment in chondrocyte differentiation with *Pten* ablation in chondrocytes leading to dyschondroplasia thereby suggesting a mechanism through which the growth plate phenotype occurs.

A third study (Hsieh *et al.* 2009) that was also a chondrocyte specific model, used the *Col2cre* crossed to *Pten* flox/flox mice. These mice have only exon 5 of *Pten* floxed, but on utilization of the Cre showed a similar loss in PTEN protein levels in a manner identical to other *Pten* floxed mice (Lesche *et al.* 2002). The chondrocyte-specific knockout of *Pten* in this case also showed increased chondrocyte differentiation and growth plate defects similar to the above-mentioned studies. In addition, the long bones of these mice had an increase in marrow adipogenesis with lipoma formation. Through the use of a Rosa 26 reporter expression to study the Cre expression the authors raised the possibility that *Pten* also plays a role in regulating adipocyte formation.

The increased adipocytic phenotype can be explained by the activity of the Cre targeting adipocyte precursors among MSCs leading to an increase in AKT that has been shown to be necessary for adipogenesis. The phenotypes of the different chondrocyte-specific knockouts show a reasonably similar phenotype when growth plate development is compared. But, the difference among these knockouts cannot be attributed solely to deletion of *Pten* as there was an efficient

knockout of *Pten* and activation of pAKT. On the other hand, the differences could be due to variations in the genetic background of the mice utilized for these studies. The phenotypes of the various conditional knockouts that have been reported have been summarized in Table 1.

Osteoblast-specific knockout of Pten

Deletion of *Pten* in late stage osteoblasts using a Cre that is under the control of an osteocalcin promoter which starts expression at around 17 dpc (Zhang *et al.* 2002) showed there is an accumulation (increase) of bone mass in bones that develop through both intramembranous and endochondral ossification. This would suggest a cell-autonomous effect of loss of PTEN on bone development. This potential mechanism was studied with calvarial osteoblasts and the authors observed a decrease in osteoblast apoptosis and an increase in MTOR signaling pathway molecules in cells from PTEN-null mice (Liu *et al.* 2007).

Another study using a conditional knockout approach was performed by *Dermo1*Cre to delete *Pten* in osteoprogenitors (Guntur *et al.* 2011). *Dermo1* or *twist2* Cre starts expression at around 9.5 dpc and targets mesenchymal cells so it works on precursors that are destined to become skeletal cells (Li *et al.* 1995, Yu *et al.* 2003). Loss of *Pten* in osteoprogenitors resulted in an increase in osteoblast progenitor proliferation specifically in the perichondrium and was accompanied by 1) enhanced FGF signaling; 2) increased *Fgf18* expression; and 3) a decrease in Sprouty2, an inhibitor of FGF signaling, which when activated increases perichondrial osteoprogenitor proliferation and differentiation. Not surprisingly, the expression of Sprouty2, at the transcriptional level, is regulated by the FoxO group of transcriptional factors. For example, we found that overexpression of FOXO3 in C3H10T1/2 cells leads to suppression of *Fgf18* expression. FGF signaling in turn activates GLI2 activity via downstream effectors, leading to osteoblast differentiation (summarized in Table 1).

Marrow adipogenesis is an alternative pathway for mesenchymal stromal cell differentiation and is dependent on the activation of several critical transcription factors, including *Pparγ*. As noted, chondrocyte-specific deletion of *Pten* resulted in the appearance of marrow adipocytes (Hsieh *et al.* 2009). These findings have significant clinical implications. First, in age-related osteoporosis, osteoblast progenitor numbers are reduced while marrow adipogenesis is enhanced suggesting that lineage allocation is a critical determinant of subsequent bone turnover even in the elderly (Nuttall *et al.* 1998). Second, increased *Pparγ* activation with agents such as the TZDs, force mesenchymal progenitors into the adipocyte pathway and this results in significant bone loss and a greater risk of fracture in type II diabetes mellitus. From a therapeutic perspective, agents that selectively target lineage allocation in progenitor cells might offer promise as anabolic agents, or to prevent age-related marrow adipogenesis or to set the stage for more robust skeletal formation under the appropriate circumstances.

The latter is provocative and may be critical in a number of scenarios. As shown, the *Pten* and the PI3K signaling pathway clearly play an important role in regulating tissue-specific progenitor cell fate (Yilmaz *et al.* 2006, Hill & Wu 2009). This is further supported by findings that an increase in the activity of the FOXO is associated with a loss in bone mass and an increase in marrow adipogenesis (Moerman *et al.* 2004, Almeida *et al.* 2007). Additional evidence from *Akt1/Akt2* knockout mice shows that in the absence of AKT there is an inhibition of adipocyte differentiation and that AKT is important for adipogenesis by inducing *Pparγ* expression. On the other hand, it could be argued that the increase in proliferation in *Pten* deleted cells maintains precursor cells as an immature population allowing for expansion of marrow adipocytes under the appropriate conditions. Experimental studies in our laboratory suggest that marrow adipogenesis is an early feature of injury models such as irradiation post bone marrow transplant or during the first 48 h post fracture. Maintenance of precursor cells in their 'stem-like' state by marrow adipocytes could be a critically important function by acting as 'placeholders' to allow progenitor cells time to be primed before differentiation. Ultimately, though, in the bone marrow irradiation/transplant model, adipocytes disappear either by apoptosis or by conversion to a new cell type; this is subsequently followed by rapid hematopoietic repopulation. Thus, fully understanding lineage allocation and the role of PI3K in this process is an important clinical and translational goal.

PI3K, Pten signaling and intramembranous ossification

All the above studies examined the role of PI3K signaling during endochondral ossification. However, changes in PI3K signaling during intramembranous ossification have not been studied in a systematic manner. For example, examination of the *Akt1/Akt2* knockout animals shows a clear defect in intramembranous ossification that could be attributed to PI3K signaling regulating RUNX2. One study utilized the *Pten* flox/flox/*col2a1* Cre as a model to study the effect of variation of phenotypes on the shape of the skull. As the base of the skull develops through endochondral ossification the authors observed a shortening of the skull base (Hallgrímsson *et al.* 2007). Furthermore, the osteocalcin Cre *Pten* conditional knockout exhibit increased calvarial bone density suggesting that the *Pten* knockout has a cell-autonomous effect on cranial bone development. In another study where AKT signaling was modulated in the skeleton by expressing a dnAKT (dominant negative) form under the control of a *col2a1* promoter, skull base formation (i.e. shortening of the skull base) was affected, a finding which is nearly identical to the effect of *Pten* deletion on the skull base (Rokutanda *et al.* 2009). Studying the effect of PI3K signaling or its downstream effectors during intramembranous ossification using mouse models that have lesions in the PI3K signaling

effectors might provide novel information about skull development and provide new therapeutic avenues to treat pathological conditions, particularly in respect to craniofacial disorders.

Diverse effects of the PI3K pathway

One of the most exciting developments in skeletal biology has been the establishment of the skeleton as a modulator of glucose and metabolic homeostasis (Lee *et al.* 2007, Ferron *et al.* 2010, Clemens & Karsenty 2011). For example, a recent study in which FOXO1 was deleted only in osteoblasts revealed that not only was bone mass affected but so was glucose metabolism (Rached *et al.* 2010a,b). Though *Pten* specific knockouts should inactivate FOXO1 in chondrocytes and osteoblasts, and thereby lead to the metabolic effects observed with the osteoblast-specific deletion of FOXO1, none of the studies to date have addressed this question. Furthermore, osteoprogenitor-specific deletion of *Pten* led to FGF signaling-mediated activation of MAPK signaling showing that this arm of the signaling mechanism downstream of tyrosine kinase receptors like FGFR is also activated. Thus, studying the energy sensor kinase MTOR, the other major target of AKT (Fingar & Blenis 2004), and its role in osteoblast differentiation would be provocative. For example, the *Pten* conditional knockout using an osteocalcin Cre by Liu *et al.* clearly showed that deletion in calvarial osteoblasts was associated with an increase in MTOR activity. In addition, the study by Ford-Hutchinson *et al.* (2007) revealed an increase in pP70S6K (a downstream direct target of MTOR) in the growth plate when the chondrocytes were PTEN-null. The only studies that have directly addressed these issues however, have utilized preosteoblastic cells (MC3T3E1), rats and mouse *ex vivo* organ cultures to inhibit MTOR using rapamycin (Alvarez-Garcia *et al.* 2007, Phornphutkul *et al.* 2008, Singha *et al.* 2008, Rokutanda *et al.* 2009, Sanchez & He 2009). Therefore, it would be essential to study the role of MTOR signaling both in the context of loss of *Pten* or downstream of activation PI3K signaling during osteogenesis. In that respect, a recent study showed that loss of IGF-binding protein 2 (IGFBP2) in male mice led to an increase in PTEN protein expression in bone marrow stromal cells and calvarial osteoblasts (Demambro *et al.* 2008, Kawai *et al.* 2011). This was accompanied by an increase in marrow adiposity suggesting that enhanced *Pten* activity played a role in regulating bone development by controlling MSC fate. Not surprisingly, *Igfbp2*^{-/-} mice develop mild insulin resistance and glucose intolerance with age. In that same model, treatment of the *Igfbp2*^{-/-} mice with a short polypeptide for the heparin-binding domain of the IGFBP2 molecule rescued the low bone mass phenotype and markedly suppressed PTEN expression (Kawai *et al.* 2011). Though there have been no lesions in humans that have been identified that affect PI3K signaling directly, syndromes like GH deficiency that cause Laron's syndrome

and skeletal deficiencies, affect IGF1 levels in the body and thereby potentially modulate PI3K signaling. Moreover, PTH 1–34 and 1–84 are two peptides approved for the treatment of postmenopausal osteoporosis by enhancing bone formation (Neer *et al.* 2001, Greenspan *et al.* 2007). Although the primary mechanisms that underlie the skeletal anabolic activity of PTH are not totally defined, activation of the AKT/PI3K pathway via induction of IGF1 in osteoblasts is one pathway proven to be operative when PTH is administered.

Conclusions

In sum, we have shown that PTEN and PI3K are part of a critical nodal pathway in skeletal development and bone turnover. Neither the growth factor/PI3K nor Wnt/ β -catenin pathways exist in a vacuum; rather these two networks are integrated with other signaling networks. The context-specific nature of these pathways and their overlap lead to a significant level of complexity that will require further studies. Notwithstanding, experimental attempts to modify PI3K signaling to favorably impact progenitor recruitment and skeletal acquisition, or reduce the severity of craniofacial disorders, represent a major therapeutic challenge that could be extremely rewarding.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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