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Periostin action in bone

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ABSTRACT

Periostin is a highly conserved matricellular protein that shares close homology with the insect cell adhesion molecule fasciclin 1. Periostin is expressed in a broad range of tissues including the skeleton, where it serves both as a structural molecule of the bone matrix and a signaling molecule through integrin receptors and Wnt-beta-catenin pathways whereby it stimulates osteoblast functions and bone formation. The development of periostin null mice has allowed to elucidate the crucial role of periostin on dentinogenesis and osteogenesis, as well as on the skeletal response to mechanical loading and parathyroid hormone. The use of circulating periostin as a potential clinical biomarker has been explored in different non skeletal conditions. These include cancers and more specifically in the metastasis process, respiratory diseases such as asthma, kidney failure, renal injury and cardiac infarction. In postmenopausal osteoporosis, serum levels have been shown to predict the risk of fracture-more specifically non-vertebral- independently of bone mineral density. Because of its preferential localization in cortical bone and periosteal tissue, it can be speculated that serum periostin may be a marker of cortical bone metabolism, although additional studies are clearly needed.

1. Introduction

The processes that control bone homeostasis in response to hormonal influences, mechanical loading and tissue injury are complex and involve a myriad of growth factors, cytokines and other molecules expressed in the bone environment. The ultimate result of these physiological processes, namely the maintenance, gain or loss of bone mass and strength depends on the degree of coupling between osteoblasts, – the bone forming cell-, and osteoclasts, -the bone resorbing cell-, and their coordination by osteocytes, the end-product of osteoblasts differentiation. The latter are increasingly considered as the “astrocytes” of bone, which not only control bone modeling, i.e. the activity of bone forming lining cells without prior resorption, but also bone remodeling, i.e. bone formation following bone resorption (Bonewald, 2011). As the cellular effector of the skeletal mechanostat, osteocytes mainly repress bone formation by the production of sclerostin, a main inhibitor of Wnt-beta-catenin signaling in osteoblasts (Baron and Kneissel, 2013). This signaling pathway has recently been found to also play a role in osteocytes themselves, notably to control their expression of OPG, a main inhibitor of bone resorption (Kramer et al., 2010a). More recently, another molecule primarily expressed in bone by osteocytes, and capable to modulate Wnt-beta-catenin signaling has emerged as another key player in bone homeostasis: periostin, which pleiotropic functions in bone, regulation and potential use as a novel (cortical) bone marker are reviewed here (Table 1).

2. Expression, structure and function of periostin

Periostin (Postn) is a 90kD extracellular matrix protein of 836 aa (in humans), originally named osteoblast specific factor 2 (OSF-2) when it was first cloned from a cDNA library prepared from the mouse osteoblastic cell line MC3T3-E1 (Takeshita et al., 1993). Although the current denomination derives from its expression in the periosteum of long bones, periostin is broadly expressed, with highest levels in the aorta, stomach, lower gastrointestinal tract, placenta, uterus, thyroid tissue and breast. It is particularly expressed during ontogenesis and in adult connective tissues submitted to mechanical stimulation (stretch), such as heart valves, skin, periodontal ligaments, tendons and bones. Postn gene expression is up-regulated by platelet-derived growth factor (PDGF) and basic fibroblast growth factors (FGF) in cancer lines through different pathways including PI3K and p38MAPK. In bone,

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Table 1
Functions of periostin in bone homeostasis. Immature crosslinks (dihydroxylysinonorleucine, DHLNL and hydroxylysinonorleucine, HLNL) and mature crosslinks (pyridinoline, PYD and deoxypyridinoline, DPD) in bone extracts.

Matricellular periostin	Postn$^{-/-}$ mice
Circulating levels are higher in men than in women	At the bone tissue level there are no specific sex difference in *Postn*$^{-/-}$ mice
Linked to fibronectin, tenascin-C and collagen 1	↓ osteoblast proliferation, mineralization and attachment on bone matrix
Expressed in lining cells, osteoblasts and osteocytes	↓↓ femoral BMD
Responsive to PTH, TGF-β and mechanical loading	↓↓ cortical bone volume and thickness
Binding to αVβ3 integrin receptors	↓ trabecular bone volume on tissue volume
Signaling through AKT/FAK and Wnt-beta-catenin pathways	↓ bone formation (MAR) mainly at the periosteal surface
Down-regulates Sost	Altered collagen organization and ↓ levels of crosslinks (PYD/DPD, DHLNL/HLNL)
May upregulate OPG	↑ microcracks accumulation on repair ratio
Effects on osteoblasts migration, proliferation and differentiation	Alter osteocytic network organization
Effects on osteocytes apoptosis	↓ extrinsic bone strength and intrinsic mechanical properties (Hardness)
Direct effects on osteoclasts remains to be investigated	

Postn is transcriptionally regulated by Twist, RUNX2, and C-Fos/AP1. Its expression levels are maximal in the periosteum and osteocytes (see below) and further controlled by mechanical stimuli, hormones (PTH), growth factors (TGF-β, BMP2) and cytokines (TNF-α, IL-4, IL-13, and likely PDGF), all known to have important roles in the determination and/or regulation of bone homeostasis (Merle and Garnero, 2012).

Periostin is constituted of a signal sequence, followed by an Emilin-like domain rich in cysteine, four repeated fasiclin 1, and a C terminal variable domain which gives 7 splice variants human isoforms. Periostin sequence is highly conserved during evolution. Periostin can be modified post-translationally by a vitamin K dependent enzyme which changes glutamic residues (Glu) to γ-carboxyglutamate (Gla) (Coutu et al., 2008), because Postn contains 28 potential sites of carboxylation. Whether this gamma carboxylation is present in bone and alters the function of Postn, as it is the case for osteocalcin, is currently unknown.

Periostin has at least two major functions, one in fibrillogenesis which occurs in the matrix and another in cell migration (Kudo, 2011). Thus, in bone, periostin enhances the proteolytic activation of lysyl oxidase which is required for collagen cross-linking. This activity is accomplished by the ability of periostin to directly interact with type I collagen, fibronectin, Notch1, tenascin-C and BMP-1. In addition, periostin activates integrin-mediated signaling (see below), thereby promoting cell adhesion and motility by activation of the actin/myosin contractile machinery (Gillan et al., 2002; Shimazaki et al., 2008) (Fig. 1).

3. Periostin-activated signaling pathways

Current knowledge about periostin signaling mainly comes from studies aiming to elucidate its role in tumorigenesis and the interaction between cancer cells and their metastatic niche (Ruan et al., 2009). Periostin interacts primarily with integrin receptors αVβ3 & αVβ5 which initiate a crosstalk with receptor tyrosine kinases (RTKs), such as EGFR and VEGF (Ghatak et al., 2014) (Fig. 1). Periostin binding to integrins activates AKT/PKB and the FAK-mediated signaling pathways, while inhibiting GSK3β, -a main regulator of the Wnt- β -catenin signaling pathway-, through PiP3 and/or AKT (Morra and Moch, 2011). Periostin is also capable to indirectly decrease β-catenin degradation by blocking PTEN, an activator of β-catenin degradation (Tkatchenko et al., 2009). Thus periostin was shown to activate Wnt-β catenin signaling in primary osteoblastic cell cultures and in TOPGAL reporter mice which uses a β-galactosidase reporter gene (LacZ) under the control of multimerized TCF binding sites (Fig. 1). Moreover in epithelial cells periostin was shown to activate mTOR signaling through phosphorylation of AKT, which mediates mitogenesis and migration of these cells (Rosselli-Murai et al., 2013).

4. Periostin functions in bone cells

In MC3T3-E11 osteoblastic cells, overexpression of Postn increases cell proliferation and differentiation (Horiuchi et al., 1999). Consistent with these in vitro observations, injection of an adenovirus overexpressing periostin, increased bone formation rate and bone mass in rats (Zhu et al., 2009). Conversely, Postn deletion results in a defective attachment of osteoblasts to the bone matrix, which affects their differentiation into mature osteoblasts as shown by a severe reduction of the expression of type I collagen, osteocalcin, osteopontin and alkaline phosphatase, as well as a decreased mineralization process in vitro (Litvin et al., 2004; Bonnet et al., 2012). In the UMR-106 osteosarcoma cell line, periostin inhibits sclerostin gene (Sost) expression, whereas periostin neutralizing antibodies markedly reduced Sost inhibition by PTH. Moreover, blocking αVβ3 integrin receptors prevented sclerostin inhibition by periostin, indicating that in addition to its effects on osteoblasts, periostin may have autocrine functions on sclerostin-expressing cells (Bonnet et al., 2012). Co-immunoprecipitation experiments further suggest that periostin may directly interact with sclerostin to inhibit sclerostin antagonistic activity on WNT signaling and thereby it promotes bone formation by MC3T3 in response to BMP and Wnt ligands (Chan et al., 2008).

In the IDG-W3 cell line, Postn expression increased with the differentiation of the late osteoblast into osteocytes (Fig. 2A). In these cells, Postn levels were stimulated upon exposure to PTH (Fig. 2B). Similarly, Postn is rapidly up-regulated in response to PTH in osteocytes *in vivo* (Bonnet et al., 2012). Thus PTH-stimulated Postn expression could regulate Sost and possibly Wnt-β catenin regulated genes in osteocytes, such as OPG, by an autocrine mechanism. It remains to be elucidated whether periostin affects other osteocytic cell functions, such as osteocytic osteolysis during lactation, as well as the survival of osteocytes. In this context, it is interesting to note that 3 months administration of high-dose zoledronate decreased the number of osteocytes in both wild type and Postn KO mice, but increased the number of empty osteocyte lacunae only in the latter, suggesting an anti-apoptotic effect of periostin in osteocytes (Bonnet et al., 2013a).

Under physiological condition, immunohistochemical staining for periostin in bone does not reveal any significant expression in osteoclasts. However recent in vitro studies suggest that Postn is also expressed at low levels in osteoclasts from mouse long bones differentiated in vitro (Merle et al., 2014). Whether periostin expressed by osteoclasts represents yet another coupling factor with osteoblasts and/or exerts autocrine functions in these cells (which actually express integrin receptors to which periostin could

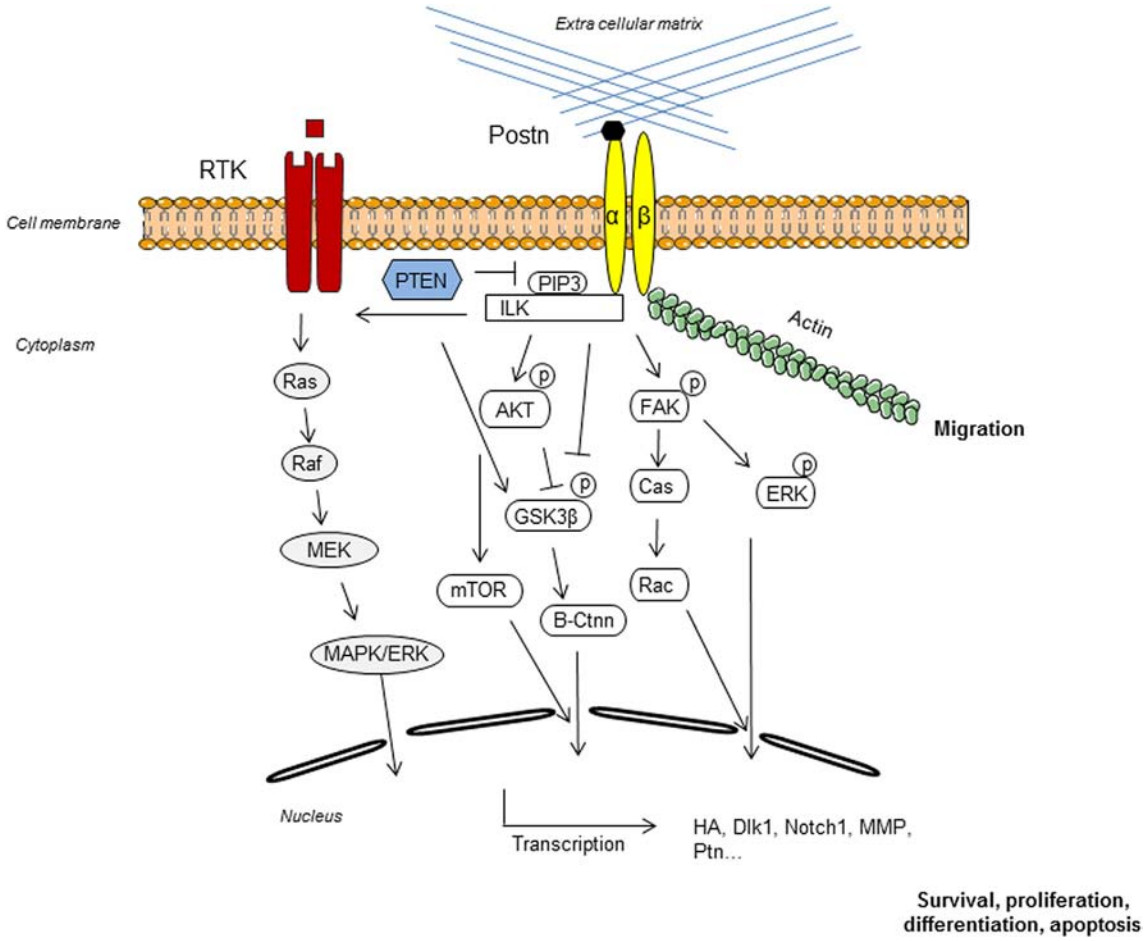


Fig. 1. Signaling pathways of periostin, adapted from Morra et al. (Morra and Moch, 2011). Periostin binding to integrins initiates integrins signaling and promotes the recruitment of tyrosine kinases receptor (RTK) at the plasma membrane. This crosstalk activates Ras/MAPK/ERK, Akt/PKB and the FAK mediated signaling pathway. It is important to point-out that the control of GSK-3β phosphorylation by Akt is more and more highlighted as an important regulator of β catenin (βCtnn) signaling pathways. The convergence of these pathways results in the activation of select transcription factors such Notch1, β-catenin …, most important pathway in osteoblast/osteocyte; which control cell survival, proliferation, migration, differentiation and apoptosis. Mitogen-activated protein kinase (MAPK); Focal adhesion kinase (FAK); phosphatidylinositol (Kramer et al., 2010a; Takeshita et al., 1993; Merle and Garnero, 2012)-trisphosphate (PIP3); Interleukin kinase (ILK); mammalian target of rapamycin (mTOR).

bind) remains to be elucidated (also see "lessons from Postn KO mice", below).

5. Periostin functions in fracture healing

In rodent models of transverse tibial diaphysis fracture, periostin mRNA and protein levels are rapidly upregulated during fracture healing, particularly in proliferating osteoblast cells of the periosteum as well as in undifferentiated mesenchymal cells. Periostin has been demonstrated to be involved in various phases of bone repair: (Bonewald, 2011) early on during inflammation and angiogenesis; (Baron and Kneissel, 2013) on the recruitment of osteoprogenitors into the callus; (Kramer et al., 2010a) in the early stages of osteoblast differentiation and bone formation. In a calvarial bone defect model, periostin has been shown to increase numbers of CD-31-positive endothelial cells and α-SMA-positive arterioles within the defect, indicating an effect on angiogenesis (Heo et al., 2015; Thorfve et al., 2014). The role of periostin in angiogenesis has been confirmed in other tissues, namely keloids (overgrowth of granulation tissue) through the activation of the ERK and focal adhesion kinase pathway, as well as the upregulated expression of VEGF and angiopoietin-1. Indeed there was a good correlation between periostin levels and blood vessel density in keloid tissue ($r = 0.711$, $p < 0.01$) (Zhang et al., 2015). To our knowledge this interaction has never been directly investigated in long bones. Considering the important role of vascularization in bone metabolism and bone repair, a better understanding of the interaction between periostin and angiogenesis might expand our knowledge on bone remodeling response to gravity, PTH and fracture repair.

6. Lessons from the Post KO mice

6.1. Skeletal phenotype of Postn KO mice

Periostin functions have now been extensively investigated thanks to the development of Postn-deficient mice (Rios et al., 2005). At first periostin was shown to be expressed in the periodontal ligament, where it acts as a critical regulator of tooth formation and maintenance. Experimental tooth movement has been shown to increase periodontal fibroblast proliferation in association with increased periostin mRNA and protein expression (Wilde et al., 2003). Accordingly, Postn-deficient mice show severe alterations in tooth eruption, resulting from a failure to digest collagen fibers in the shear zone of the periodontal ligament (Kii et al., 2006). As a consequence, the enamel and dentin of the incisors is

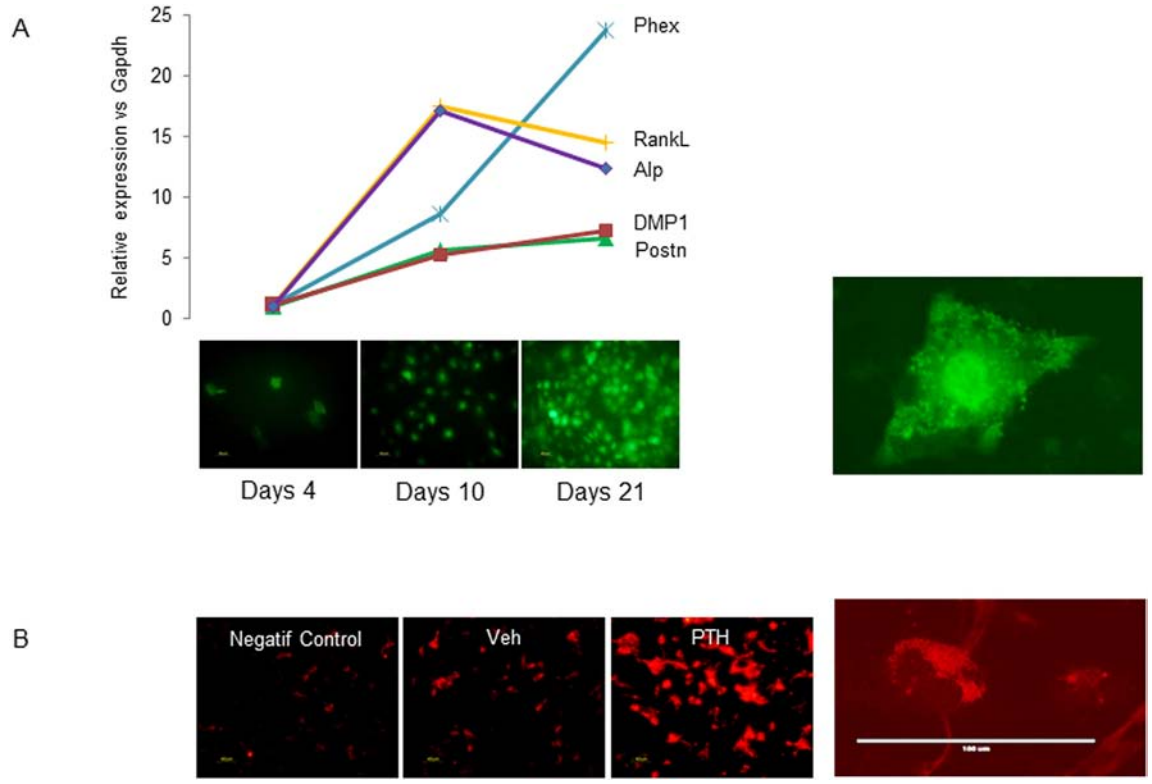


Fig. 2. Periostin expression in IDG-W3 (for Immortomouse/Dmp1-GFP-SW3) late osteoblast/osteocyte. (A) Relative expression of periostin along osteoblast differentiation into osteocyte which can be followed in live through GFP. (B) Immunostainning of periostin in IDG-W3 at days 21 after 24 h of vehicle (Veh) or PTH.

compressed and disorganized and the jaw bone is altered (Rios et al., 2005). More specifically, the crestal alveolar bone is decreased, and the periodontal ligament appeared enlarged. Basal bone, which does not belong to the periodontal tissue, was also affected as fibrous areas were increased in Postn KO (Bonnet et al., 2013a).

Postn KO mice exhibit a low bone mass and cortical architecture, with a reduced cortical bone volume and a decrease in bone strength in young adult mice, which is accentuated with aging (Rios et al., 2005; Bonnet et al., 2009). The features are primarily associated with reduced bone formation, particularly at periosteal surfaces, with a marked decrease in MAR suggesting an effect mainly on the activity of lining cells and cortical expansion. Taken together with the predominant expression of periostin in osteocytes and the periosteum, these observations also suggested a role of periostin in modeling-based responses to mechanical loading (i.e. the mechanostat, see below).

In addition, in Postn KO mice at one year of age, there is evidence of increased intracortical bone remodeling (Fig. 3). Postn KO mice did not exhibit changes in TRAP5b activity or osteoclast number in long bones. However, osteoclast numbers were increased in their femur in response to unloading (Gerbaix et al., 2015a) and intermittent PTH (see below), as well as in alveolar bone (jaw) (Bonnet et al., 2013a). Moreover, wild type primary osteoclasts cultured with primary osteoblasts from Postn KO mice show a higher number and activity of osteoclasts as compared to co-cultures with wild-type osteoblasts. In turn adding rPostn to osteoclastic cultures decreased the number of osteoclasts, as well as their activity (Bonnet and Ferrari, 2015).

Recently mice deficient for Wnt16 have revealed the unique functions of this molecule on the skeleton as these mice were characterized by decreased cortical, but not trabecular, bone mass and strength. Similar to Postn KO mice, Wnt16 KO mice present decrease in bone formation and mechanical responses at the periosteum and increased bone resorption endocortically (Movérare-Skrtic et al., 2014). Interestingly, in these mice, Postn expression is massively down-regulated (Wergedal et al., 2015).

Finally, Postn KO mice also exhibit some alterations in bone material properties, including reduced collagen cross-linking with an enlargement of collagen fibers, lower levels of mineralization, and increased levels of microdamage (Bonnet et al., 2013b). The orientation of osteocytic lacunae, which normally appears in concentric layers parallel to the cortical bone surface, was also disorganized (Fig. 3).

6.2. Roles of periostin in the bone biomechanical responses

In wild type mice, axial compression of the tibia increases Postn mRNA and protein levels in bone (Bonnet et al., 2009). These changes are both spatially and temporally related to the inhibition of Sost expression and the increase in bone forming indices. Interestingly, periostin is preferentially expressed in the outer layers of the cortex and close to surfaces exhibiting the greatest increase in bone formation (Bonnet et al., 2009). These findings suggest that periostin may serve as a molecular conduit to concentrate biomechanical signals to the outer surfaces of bone, where gains in mass would have the most favorable impact on bone strength. Accordingly, the cortical bone response to both axial compression and treadmill exercise was markedly blunted in Postn KO mice. In these mice, Sost was overexpressed and was not inhibited following loading. In turn, sclerostin neutralizing Ab partially rescued the biomechanical response to loading in Postn KO mice. These results argue for an additional role of periostin on osteoblast independently of sclerostin signaling pathway.

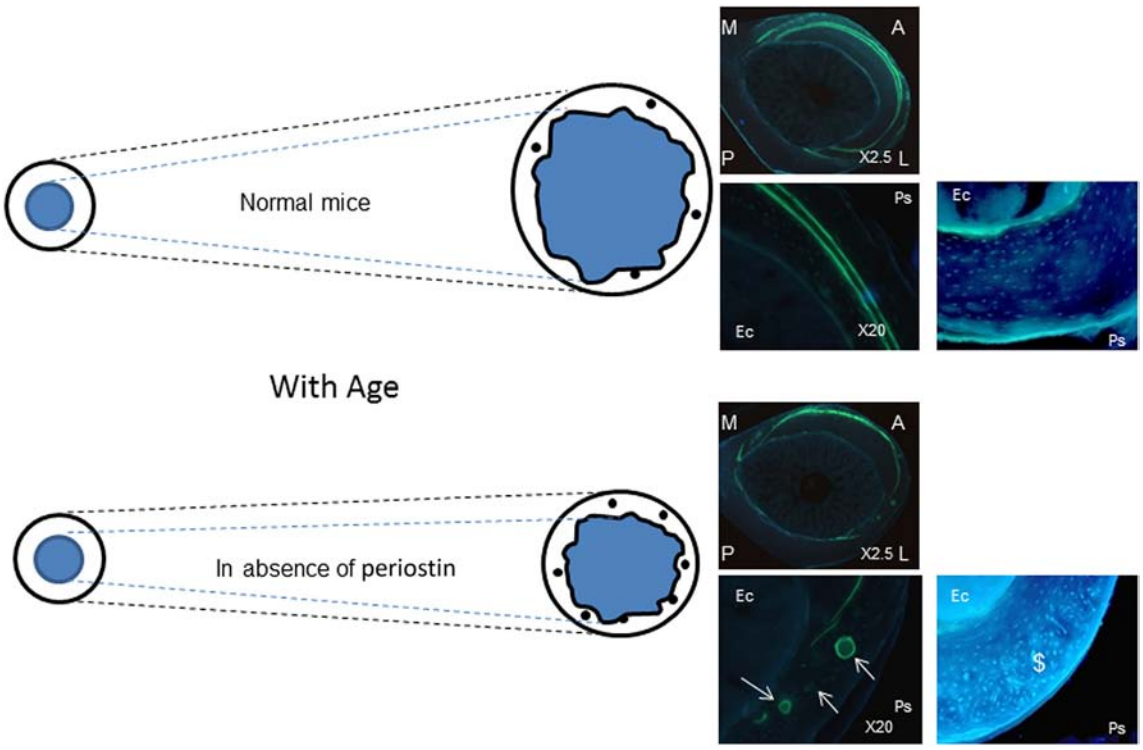


Fig. 3. Illustration of bone shape in absence of periostin with aging. A. Under normal aging despite periosteal (Ps) apposition exceed endocortical (Ec) resorption and therefore the external diameter increase, the cortex become thicker due to an increase in endo and intra-cortical resorption. It is interesting to note that bone formation is note uniform all around the bone but follow the mechanical distribution with a high strain on the antero-lateral (A-L) surface vs postero-medial (M-P) surface. In absence of periostin, double calcein injection indicates a decrease in mineral apposition rate and lower surface of labeling. Moreover, we can detect an increase in intra-cortical remodeling as indicated by calcein labeling in the pore indicating a role of periostin not only in modeling activity (indicate by arrow). We also report a disorganized osteocyte lacunae in the third external part of the cortex (indicate by $). Thereby the most striking phenotype is a small outer diameter with a low Ct.BV and Ct.Th vs wild-type.

Three other publications independently confirmed the role of Postn in response to exercise and its interaction with sclerostin expression. The first one confirmed an increase of periostin expression in osteoblasts and osteocytes in response to 3–12 weeks of intense exercise, which is a model of work-related musculoskeletal overload (Rani et al., 2009). The second showed that periosteal bone formation and improved bone microarchitecture in response to exercise was abolished in mice deficient for the microRNA miR17-92, in which the expression of *Elk3*, *Runx2* and *Postn* remained low (Mohan et al., 2015). The last one demonstrated that alteration of the skeleton and lower alveolar bone volumes in Postn KO mice were attributed to an overexpression of sclerostin. Moreover, by crossing Postn KO with Sost KO mice, the altered alveolar bone phenotype of the former was normalized (Ren et al., 2015). Altogether these experiments demonstrate that Postn expression and its induction by mechanical forces occur upstream of sclerostin inhibition in the bone mechanostatic pathway. To confirm that periostin mediates the bone biomechanical response through Wnt-β catenin (Ctnnβ) activation within the osteocyte, osteocyte-specific Ctnnβ1/Postn double haploinsufficient mice were generated. In these mice, the cortical bone response to mechanical stimulation was altered to a similar degree as in Postn KO mice (Bonnet and Ferrari, 2013). More recently the interactions between periostin and the sclerostin-Wnt/β-catenin signaling pathway has been confirmed and targeted as strategy to prevent neurectomy-induced bone loss (Lv et al., 2015).

Considering the functions of periostin on bone matrix structure and material properties (above Table 1), as well as on osteocytes functions, it was expected to also be involved in bone resistance to stress fractures and injury repair. Upon fatigue loading of the tibia, Postn KO mice presented more cracks than wild-type littermate, and these cracks persisted longer, arguing for some additional functions of periostin in the damage remodeling process. This is likely to occur through the stimulation of OPG expression by the activation of β-catenin signaling in osteocytes (see above) (Kramer et al., 2010a). Moreover, and consistent with periostin role on modeling-based bone formation, the callus bone volume in response to fatigue loading was diminished in Postn KO mice, resulting in lower bone strength (Bonnet et al., 2013b).

There is also some evidence that periostin plays a role during mechanical unloading, a process known to induce bone loss through an unbalanced process of increased bone resorption and decreased bone formation (Vico and Alexandre, 1992; Vico et al., 1998). Hence tail-suspension results in deterioration of trabecular and cortical structures as well as suppression of periosteal and endocortical bone formation. These changes are accompanied by an early and persistent downregulation of Postn expression and a concomitant upregulation of Sost (at 7 days), followed by Dkk1 (at 21 days), whereas the Rankl/Opg expression ratio increases progressively. Consistent with a role for Postn in the regulation of Sost (see above), Sost mRNA levels do not significantly increase in response to unloading in Postn deficient mice (Gerbaix et al., 2015b). In contrast, Dkk1 increases similarly to wild type, and changes in Opg and RankL are also unaffected, indicating that in these conditions, Postn selectively regulates Sost expression in osteocytes. As a result, low levels of periosteal bone formation rate were maintained in tail-suspended Postn KO mice, which did not lose further cortical bone mass. In contrast, trabecular bone loss in response to unloading persisted even in the absence of Postn, again supporting the specific role of this molecule on cortical bone

homeostasis.

7. Role of periostin in response to intermittent PTH

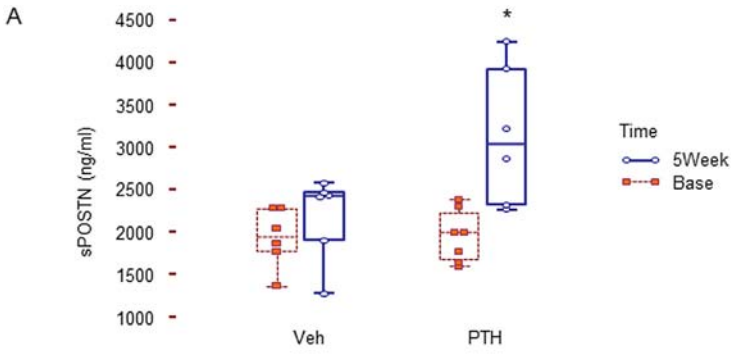
Postn expression is highly upregulated by PTH in human osteoblasts, in mice, as well as in bone extracts from patients with primary hyperparathyroidism (Bianchi and Ferrari, 2009; Onyia et al., 2005; Li et al., 2007; Reppe et al., 2006; Fortunati et al., 2010). Serum periostin levels are also significantly increased in response to intermittent PTH (iPTH) in mice, and correlate with cortical structure parameters, independently of P1NP and TRAP (Fig. 4A–B). In Postn KO mice, iPTH did not increase cortical bone forming indices nor cortical bone volume or thickness (Bonnet et al., 2012). In contrast, PTH-stimulated bone formation on trabecular surfaces was maintained in absence of Postn. Hence, absence of Postn leads to compartment-specific alterations in the response to iPTH.

Similar to mechanical loading, the bone forming effects of iPTH appear to be at least partially mediated through the inhibition of sclerostin. PTH-stimulated increases in bone mass and structure are blunted in mice over-expressing Sost, as well as in Sost-deficient mice (Robling et al., 2011; Kramer et al., 2010b). Sclerostin protein expression is not suppressed by iPTH in the bones of Postn KO mice. Consistent with these *in vivo* observations, in UMR-106 osteoblast-like cells, periostin neutralizing antibodies partially prevented the PTH-induced downregulation of Sost and its transcription factor Mef2C (Bonnet et al., 2012). However, sclerostin-blocking antibodies do not fully restore iPTH effects in Postn KO mice, which differs from the full rescue observed in these animals when subjected to mechanical loading (Bonnet et al., 2009). These observations further suggest that PTH-stimulated Postn expression may exert its effects on cortical bone formation not only by down-regulating Sost but also by directly stimulating lining cells. Consistent with this hypothesis, the PTH-stimulated activation of β-catenin signaling observed in osteocytes and osteoblasts at the endocortical and periosteum surfaces using TOPGAL reporter mice was virtually suppressed in Postn KO mice (Bonnet et al., 2012). These results identify periostin as a regulator of the Sost/Wnt/β-catenin signaling pathway not only in response to mechanical loading but also in the anabolic response to PTH. Finally, periostin can regulate bone formation through a direct stimulation on Wnt-β catenin, and via indirect stimulation of Wnt-β catenin through sclerostin inhibition, as well as through non-canonical Wnt signaling pathways, as also previously demonstrated in epithelial cells (Haertel-Wiesmann et al., 2000).

8. Periostin as a potential clinical biomarker

Periostin is a secreted protein, it can thus be measured in peripheral biological fluids including serum. Several clinical studies have been performed using various researches ELISA based on polyclonal or monoclonal antibodies with different specificity. More recently two commercially available immunoassays have been developed. The assay from USCN (China), uses a polyclonal antibody raised against the FAS-1 domain of Postn and is expected to detect all Postn isforms (Rousseau et al., 2015).

A second more recent ELISA from Adipogen (Switzerland) uses two monoclonal antibodies of unknown specificity and it remains unclear which molecular forms it detects. Because research and commercial assays are not standardized it is difficult to compare the results between studies, and this could influence the performance of the data. Using these ELISA, it has been shown that in postmenopausal women serum periostin did not (Anastasilakis et al., 2014; Rousseau et al., 2014; Bonnet et al., 2015) or only weakly (Kim et al., 2015) correlate with spine and hip areal BMD. Similarly serum Postn was not or only slightly associated with conventional markers of bone formation and bone resorption in the same clinical studies. These data indicate that when assayed in serum, Postn levels reflect biological processes which are different from those captured by static (BMD) or dynamic (bone markers) indices of bone metabolism. (Rousseau et al., 2014; Bonnet et al., 2015). In a cohort of healthy elderly men and women, a positive correlation between serum periostin and QCT assessed cortical bone thickness was observed whereas it correlated negatively with cortical porosity, although these associations were not anymore significant after adjustment for gender and age. In the same individuals, serum periostin was not correlated with trabecular bone volume fraction (BV/TV) after adjustment by P1NP, CTX, BMD,



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			Ec			Ps				
r^2	TRAP	P1NP	MAR	MPm/BPm	BFR/ BPm	MAR	MPm/ BPm	BFR/ BPm	Ct.BV	MI
sPOSTN	0.11	0.01	0.43 ***	0.28 **	0.32 **	0.31 **	0.39 ***	0.33 **	0.30 **	0.24 **
P1NP	0.01	/	0.12	0.28 *	0.24 *	0.07	0.17	0.14	0.025	0.09

Fig. 4. Correlation of sPOSTN with bone formation indices and cortical bone microarchitecture parameters, independently of bone turnover markers. (A) Five weeks of intermittent PTH significantly increased sPOSTN levels, *$p < 0.05$ vs baseline; (B) Better correlation of sPOSTN with bone formation indices and cortical microarchitecture than P1NP. Periosteum (Ps) and endocortical (Ec) surfaces.

gender or age (Bonnet et al., 2015). There was also an association of periostin levels between parents and adult offspring, with an estimated heritability of 50%, and a genetic covariance was found between serum periostin and bone microarchitecture. In a cohort of post-menopausal women, the highest quartile of serum periostin measured by the USCN assay was associated with an increased risk of all incident fractures, independently of BMD and conventional bone markers (Rousseau et al., 2014). These data were more recently confirmed in another case control study of post-menopausal women from Korean (Kim et al., 2015). It was found a positive association between serum periostin and all incident fractures independently of BMD (Kim et al., 2015). Interestingly, when vertebral and non-vertebral fractures were analyzed separately, periostin levels were associated only with the latter fracture type, which is in agreement with the predominant role of Postn on cortical bone metabolism (Bonnet et al., 2015). It should be mentioned that in this investigation serum periostin was measured by an assay which was different from that used in the two previously quoted clinical studies (Adipogen ELISA). All together these clinical data suggest that serum periostin could be a new biological marker of fracture risk, and more specifically non vertebral fracture in postmenopausal women. The fact that it is independent of both BMD and bone markers suggests that the measurement of serum periostin could be useful in combination with these two diagnostic tests to improve risk assessment. These data need however to be confirmed in larger independent studies in which these hypotheses can be tested. It has however to be remembered that periostin is not specific of bone and that serum levels represent the additional contribution of the metabolism of other tissues. It is possible that bone specific isoforms or Postn fragments could be a more sensitive index of fracture risk. In that respect, it is interesting to note that specific periostin isoforms have been described in other tissues such in fibrotic lung, heart, skin (Kühn et al., 2007; Uchida et al., 2012; Masuoka et al., 2012). For example, periostin has been shown to be expressed by skeletal myofibers in order to shape matricellular components during development, muscle regeneration and differentiation (Ozdemir et al., 2014). This function appears to be dominated by a specific periostin isoforms expressed in muscle (Ozdemir et al., 2014). A periodontal ligament-specific periostin isoform has also been described (Yamada et al., 2014), along with unique isoforms in specific cancers such non-small cell cancer (Morra et al., 2012). Compelling reasons to pursue the identification and characterization of such a molecule including its potential diagnostic utility and its potential as a therapeutic target.

9. Summary and perspectives

Experiments in vitro and in Postn KO mice have started to delineate the multiple roles of periostin on bone homeostasis, particularly in the bone modeling response to mechanical stimuli and PTH through the regulation of sclerostin expression and the WNT-β catenin signaling pathway in osteoblasts and osteocytes. Through the latter, periostin could also modulate bone remodeling by regulating the expression of OPG, which is particularly important in damage repair. Since serum periostin levels appear to decrease with age and to be lower in women, we postulate that down-regulation of Postn expression could be a mechanism to explain the accumulation of microcracks with age and more so in women (Schaffler et al., 1995), as well as the declining response to exercise in the aging skeleton. On another side, increased periostin expression could play a relatively protective role on bone mass in hyperparathyroidism and its preferential expression in the outer cortex contribute to the uneven distribution of cortical porosity in this disease. Furthermore, periostin appears to be degraded by cysteine proteases such as Cathepsin K, suggesting that its level of expression could be modulated by Cathepsin K inhibitors and thereby play a role in the maintenance of bone forming surfaces observed with certain Cathepsin K pharmacological inhibitors, such as odanacatib (Duong le, 2012; Lotinun et al., 2013). Although preliminary the clinical data suggest that serum periostin -after further improvement of the assays in terms of specificity-could become a useful biomarker for the investigation of metabolic bone diseases such as osteoporosis and joint inflammatory and non-inflammatory disorders.

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