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REVIEW

The multiple facets of periostin in bone metabolism

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Abstract Periostin is a matricellular glutamate-containing protein expressed during ontogenesis and in adult connective tissues submitted to mechanical strains including bone and, more specifically, the periosteum, periodontal ligaments, tendons, heart valves, or skin. It is also expressed in neoplastic tissues, cardiovascular and fibrotic diseases, and during wound repair. Its biological functions are extensively investigated in fields such as cardiovascular physiology or oncology. Despite its initial identification in bone, investigations of periostin functions in bone-related physiopathology are less abundant. Recently, several studies have analyzed the potential role of periostin in bone biology and suggest that periostin may be an important regulator of bone formation. The aim of this article is to provide an extensive review on the implications of periostin in bone biology and its potential use in benign and metabolic bone diseases.

Keywords Bone quality · Bone markers · Bone metabolism · Bone metastasis · Periosteum

Abbreviations

βig-H3 protein	transforming growth factor-β-inducible protein
BMP	bone morphogenetic protein
CRS	carboxylase recognition sites
dpc	days postconception
DPD	deoxypyridinoline
DHLNL	dihydroxylysinonorleucine
ECM	extracellular matrix
FAK	focal adhesion kinase
FGF	basic fibroblast growth factors
Gla	γ-carboxyglutamate
Glu	glutamate
HLNL	hydroxylysinonorleucine
LOX	lysyl oxidase
PDL	periodontal ligament
PDGF	platelet-derived growth factor
PHPT	primary hyperparathyroidism
PLF	periostin-like factor
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related peptide
PYD	pyridinoline
TNFα	tumor necrosis factor α
TRAP5b	tartrate resistant acid phosphatase

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Introduction

Originally known as osteoblast-specific factor, periostin was first identified in a mouse osteoblastic cell line as a putative cell adhesion protein for preosteoblasts [1]. It was then renamed periostin due to its preferential location in the periosteum. Periosteum covers the outer surface of bone and contributes to intramembranous bone embryogenesis and growth in diameter of bone. Although periosteum is particularly active during growth, it also plays a crucial role in adults

as it contributes to the determination of bone diameter and, thus, bone strength. Periostin expression is not restricted to bone as it is predominantly expressed in collagen-rich fibrous connective tissues subjected to constant mechanical stress, such as periodontal ligament (PDL) [2–4], heart valves [2, 5], and tendons [6]. Its expression during development prevails over that in the adult organism [7–9]. However, periostin is reexpressed in adult rats after myocardial, vascular, and skeletal muscle injuries and bone fracture [10, 11]. Periostin expression is also increased in a large variety of tumors including colon, bladder, breast, non-small cell lung cancer, head and neck, oral, and pancreas. Periostin up-regulation usually correlates with aggressiveness and/or poor survival [12]. A large body of information about periostin functions comes from investigations of cardiac development or tumor expansion. However, its preferential expression in collagen-rich tissues submitted to mechanical stress such as periosteum from development to perinatal life, as well as up-regulation during fracture healing, suggests that it may play a crucial role in bone maintenance and regeneration. The aim of this review is to discuss the role of periostin in bone metabolism.

Structure of periostin

Periostin gene has been cloned in several species (mouse, rat, chicken, bovine, xenopus, etc.) and is located at locus 13q13.3 in human and 3C in mouse. Originally cloned from both human placental and osteosarcoma tissue, periostin is highly conserved between human and mouse with 89.2% homology. The mouse periostin cDNA is 3,187 bp long and contains an 18-bp 5′ untranslated region, a 733-bp 3′ untranslated region, and an open reading frame of 2,436 bp corresponding to a protein precursor of 838 amino acids (Fig. 1). The protein is composed of a signal sequence, followed by an Emilin-like (EMI) domain rich in cysteine, 4 repeated and conserved FAS-1 domains, and a C-terminal hydrophilic and variable domain. Alternative splicing of the C-terminal domain gives rise to at least five different human isoforms [1, 13]. An additional variability caused by single nucleotide polymorphism was also reported National Center for Biotechnology Information (NCBI database). Due to the presence of FAS-1 domains, periostin belongs to the fasciclin family and shows a structure similar to the insect axon guidance fasciclin and Transforming growth factor-β-inducible protein (βig-H3) involved in cell attachment [14]. The presence of integrin binding motifs in the second and fourth FAS-1 domains suggests that periostin is implicated in cell adhesion, as these domains have been shown to mediate βig-H3 adhesion to α3β1 [13]. In addition, each FAS-1 domain is rich in glutamate residues and contains an N-terminal recognition site for the vitamin K-dependent enzyme γ-glutamyl carboxylase (γ-carboxylase recognition sites, or CRS) responsible for the post-translational modification of glutamic residues (Glu) to γ-carboxyglutamate (Gla). Periostin also possesses four putative N-glycosylation sites and a heparin-binding domain (arginine-rich consensus sequence) at its C-terminal end creating a potential binding site for glycoproteins, glycosaminoglycans, and proteoglycans [3, 15]. Finally, periostin can also form disulfide-bonded dimers through its EMI domains [16, 17].

The periostin isoforms seem to be differentially expressed during development or following stress such as infarct or neoplasm [4, 8, 9, 18, 19]. Alternative splicing of periostin gives rise to different isoforms, specific of a tissue type, a development stage or a disease. The C-terminal part of periostin is encoded by exons 15 to 21, which present themselves as six cassette exons, a to f, according to the denomination established by Horiuchi and colleagues [3]. These cassettes can be present or deleted in mature mRNA in various combinations, giving rise to different isoforms [3]. Periostin isoform 1 contains the six cassettes, whereas in isoform 3, also known as periostin-like factor, cassette e is missing [9]. Additionally, a variant lacking both b and e cassettes has been described to be preferentially expressed in the periosteum and PDL as well as in heart tissues after myocardial infarction [20]. The predicted amino acid sequence analysis of the C-terminal end identified a nuclear localization sequence implying that some isoforms will be localized in the nucleus, as it has been shown for isoform 3 [8, 9]. Even though this C-terminal hydrophilic region is devoided of known protein domains, specific roles of certain isoforms have been reported in in vitro cell invasiveness according to their differential expression patterns [19, 21]. The fact that periostin, which has been localized in the nucleus and the cytosol of cells also bears a signal sequence and is secreted, suggests that periostin isoforms can have intracellular and extracellular functions as recently reviewed [20].

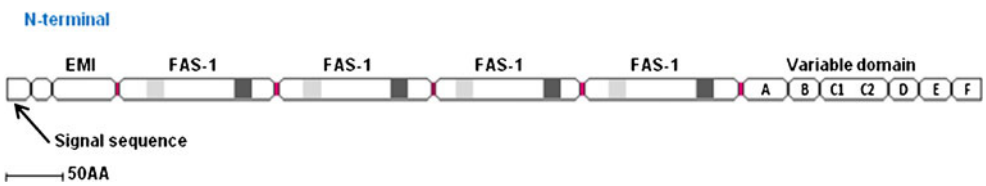

Fig. 1 Periostin protein sequence showing the signal sequence, the EMI domain, the four FAS-1 domains, and the C-terminal variable region with the six different cassettes (A–F) whose combination gives rise to different isoforms. Each FAS-1 domain contains an N-terminal recognition site for the γ-glutamyl carboxylase and a cell adhesion site

Finally, a tri-dimensional structural model for periostin has been proposed: the four FAS domains of human periostin have a secondary structure consisting of a helix-turn-helix motif, while the C-terminal region presents beta-strands [1, 13, 22].

Periostin is expressed in bone and teeth

Periostin is preferentially expressed in the periosteum, which covers a large majority of bones, and in the PDL of the teeth, a highly specialized connective tissue that links teeth to the alveolar bone. Periosteum is responsible for changes in bone diameter and cortical thickness, and bone size is related to bone strength. Periosteal apposition occurs throughout life: it is high during growth and decreases in adult life. Beyond its crucial importance during embryogenesis, periosteum is largely involved in fracture healing [23].

During embryogenesis, periostin mRNA was detected as soon as 9.5 days postconception (dpc) in mouse embryos. In developing bone, at least four protein isoforms could be detected from 12 to 19.5 dpc with an expression more or less important over time [4, 9]. Fetal development of the skeleton implies two processes: intramembranous ossification involves mesenchymal stem cells, which develop into osteoblasts, whereas in endochondral ossification, a cartilage template first formed by chondrocytes is then replaced by bone. In the developing long bone, immunolocalization showed that periostin isoforms 2 and 3 were localized to proliferating and hypertrophied chondrocytes from 12.5 dpc and all along the differentiation process. At 16.5 dpc, isoform 3 expression appeared in mesenchymal preosteoblasts cells from the periosteum and in ameloblasts and odontoblasts in developing teeth, whereas isoform 2 was primarily localized in the mesenchymal cells of the perichondrium till 19.5 dpc [4, 8]. Additionally, isoform 3 showed both nuclear and cytoplasmic expressions in accordance with the presence of a nuclear localization sequence, whereas variant 2 was only found in cytoplasm [9]. However, as the 2 antibodies used in this study have been raised against a peptide arising from cassette b or e to recognize isoform 3 or isoform 2, respectively, they should, in principle, also detect all the isoforms bearing one of these cassettes. Additionally, they will miss variants lacking cassettes b and e, described to be expressed in periosteum and PDL [8, 20]. Overall, these data show that there is a spatial and temporal expression and localization of periostin isoforms, suggesting different roles for these variant proteins during osteogenesis.

As in embryonic bone, multiple protein variants of periostin are expressed, but to a lesser extent, in neonates, young, and adult rodents bone [3, 9, 16, 24]. Periostin protein (at least isoform 3) was detected in mesenchymal cells and alkaline phosphatase expressing cells, that is, preosteoblasts and osteoblasts of the periosteum and in the osteoblasts lining trabecular bone [3, 24, 25]. Similarly, immunohistochemistry on human bone with an antibody recognizing the N-terminal part of periostin common to all variants revealed that periostin was largely expressed in the extracellular matrix (ECM) of the cambial layer of the periosteum of long bones and calvaria. Periostin mRNA was detected in periosteum cells but not in osteoblasts, osteocytes, or lining cell of the underlying bone [26]. By contrast, using two antibodies recognizing isoform 2 or 3, one group showed that these isoforms were not expressed in the periosteum nor epiphyseal plate of adult normal rat bone [27]. However, expression of both periostin variants was induced by chronic overload in the cellular periosteum, articular cartilage, osteoblasts, osteoclasts, and osteocytes in a time and spatial localization pattern [24, 27]. As mentioned earlier, these antibodies may detect more than one isoform and miss variants lacking b and e cassettes. Furthermore, in the periosteum as in the infarcted myocardium, periostin is secreted, at least in part, as a cleaved form lacking its C-terminal domain [16].

Overall, these data suggest that periostin is preferentially expressed in the periosteum at a high level during embryogenesis and bone growth. In adult, it is reexpressed after mechanical stress and fracture where bone formation is important. Further analyses are needed to localize and characterize all the isoforms to have a more complete view of the periostin functions. The concomitant stimulation of periostin and inhibition of mineralization by activin A and the presence of ectopic mineral deposit on bone in periostin KO mice strengthen the association of periostin with the early stages of osteoblast differentiation and bone formation [16, 28]. These observations are consistent with the inhibition of periostin expression by inorganic phosphate as described previously [29]. Finally, it is of interest to report the expression of periostin in fibroblast-like synoviocytes from rheumatoid arthritis [30] and in cartilage biopsies from osteoarthritis patients [31].

In situ hybridization and immunohistochemistry showed that periostin is expressed in the PDL, in dental pulp at the sites of hard/soft tissue interfaces in mouse and human tooth [32, 33]. Periostin is secreted by fibroblasts and osteoblasts in PDL. As in bone, it is noticeably absent from terminally differentiated mineralized tissues (dentin, alveolar bone) [32].

Periostin expression is transcriptionally regulated

A number of studies, especially in cancer in which periostin is largely implicated, have shown that many factors can modify periostin expression. Among the transcription factors involved in osteoblast differentiation, Runx2/cbfa1, Wnt/β-catenin, and osterix pathways are essential in the commitment of pluripotent mesenchymal cells to the

osteoblastic lineage [34]. In MC3T3-E1 osteoblast-like cells overexpressing Runx2, a factor involved in the commitment of stem cells to preosteoblasts, periostin was positively regulated, suggesting its involvement in early bone cell differentiation [35]. Periostin regulation by Wnt pathways, critical factors of bone mass regulation required in response to mechanical loading, has not yet been demonstrated in bone [36]. One of this pathway, Wnt-3, was shown to down-regulate periostin expression in an epithelial cell model [37].

Twist-1 is another transcription factor that has been shown to play both positive and negative roles in cell differentiation. Twist-1 and periostin are coexpressed by differentiating osteoblasts and fibroblasts at the osteogenic front of calvaria and at the alveolar bone surfaces in PDL [32, 38, 39]. Twist-1 homodimers can bind a response element in the 5′ flanking region of the periostin promoter and up-regulate its transcription in undifferentiated osteoblasts, whereas heterodimers binding will lead to inhibition of periostin expression and osteoblast differentiation [38, 39]. In craniosynostosis characterized by premature closure of cranial sutures due to twist-1 haploinsufficiency, there is a reduction of twist-1 level with an increase in the ratio of twist-1 homodimers versus heterodimers, leading to an up-regulation of periostin expression and a premature osteoblast differentiation and sutures closure [38]. By contrast, occlusal hypofunction in mouse PDL induced a significant but transient decrease in twist-1 and periostin expression [32]. These data suggest that there is a dynamic regulation of twist-1 dimers expression at the osteogenic front, maybe as an adaptation to environmental changes. It may result in a switch from inhibition of osteoblast differentiation to induction, associated with periostin up-regulation [38].

c-Fos/AP-1 is a transcription factor that plays an important role in bone cell proliferation and differentiation [40]. Human bone tissues from patients with fibrous dysplasia, associated with increased expression of c-Fos, expressed high levels of periostin [26]. Similarly, transgenic mice overexpressing c-Fos develop sclerotic lesions with transformed osteoblasts expressing high levels of periostin, whereas normal osteoblasts did not, suggesting that c-Fos pathway might represent one mechanism for periostin up-regulation, at least in pathological conditions resulting in altered collagen fibrillogenesis and deposition. Two potential binding sites for c-Fos/AP1 have been described in the periostin promoter [26].

Periostin expression is regulated by hormones and growth factors

Intermittent injections of parathyroid hormone (1-34) (PTH) and sex steroids are both anabolic agents that preferentially target periosteum in bone [41, 42]. Gene expression profiles studies on PHPT patients and rodents submitted to continuous infusion of PTH show increased bone turnover associated with higher periostin mRNA levels [43, 44]. In vitro, periostin expression may be directly up-regulated by PTH treatment in mouse calvaria osteoblasts [45]. As it has been shown that intermittent PTH promotes periosteal cells differentiation and early progenitors proliferation in vitro and in vivo through ERK-, bone morphogenetic protein (BMP)-, and Wnt-dependent signaling pathways, it is possible that PTH effect on periostin is mediated through one of these pathways [25]. Additionally, PTH can stimulate osteoblastogenesis through inhibition of sclerostin, an osteocyte-specific protein [46, 47]. Sclerostin, a potent antagonist of bone formation through inhibition of Wnt and BMP signaling, is also down-regulated by periostin [46, 47]. All these data suggest that PTH may influence osteoblast synthesis and subsequent bone remodeling through periostin via different ways. Finally, periostin knockdown induced a significant reduction of PTHrP mRNA and protein expression by osteoblasts in vitro, suggesting a local regulation loop [45].

In human PDL cells culture, estrogens were reported to enhance cell proliferation and periostin mRNA expression through binding to estrogen receptor B, but also to stimulate osteoblastic differentiation by increasing alkaline phosphatase activity, osteocalcin production, and mineralized nodules formation [48]. Others showed that estrogens promote undifferentiated periosteal cell proliferation but inhibit their differentiation in young rodents by attenuating PTH- or BMP-2-induced actions suggesting a possible different regulation of periostin [25]. These results are in agreement with in vivo data, showing that estrogens inhibit periosteal bone growth while being anabolic on endosteal and trabecular bone [49]. Both PTH and estrogen seem also to protect undifferentiated periosteal cells from apoptosis [25]. Leptin, an adipocyte-derived hormone, which controls body weight through its effects on food intake and energy expenditure, inhibits periostin expression while stimulating mineralization and transition from osteoblasts into preosteocytes [50].

Among growth factors and cytokines, periostin expression was shown to be regulated by several members of the TGF-β superfamily. TGF-β and BMP are well-known regulators of bone development by stimulating the differentiation of osteoblast progenitors [51, 52]. TGF-β, BMP-2, activin, and retinoic acid all stimulate periostin expression in osteoblasts [3, 28, 33, 53, 54]. Blocking TGF-β receptor activation or the noncanonical focal adhesion kinase (FAK) pathway reduced periostin mRNA expression. Additionally, FAK/src inhibition in human PDL fibroblasts reduces nuclear translocation of twist and periostin mRNA levels [33]. These data demonstrate that TGF-β can regulate periostin expression through activation of TGF-β receptors but also through FAK/src signaling pathway. FAK activates signaling molecules, leading to twist-1 translocation to the nucleus and subsequent periostin activation [33, 39]. The fact that activin and TGF-β inhibit mineralization and that periostin

expression is negatively regulated as mineralization proceeds suggests that TGF-β and activin may control the onset of mineralization, at least in part, via the up-regulation of periostin expression [28].

Periostin was shown to be up-regulated by platelet-derived growth factor (PDGF), basic fibroblast growth factors (FGF-1 and FGF-2), and angiotensin II in cancer cell lines, through different pathways including RTK/PI3K, Ras/MEK, and Ras/p38MAPK [54–57]. Their effect on periostin expression has not yet been studied in bone. However, because PDGF and FGF have been shown to stimulate bone formation in vivo, we can speculate that they also act, at least in part, through periostin regulation [58]. Tumor necrosis factor α increased periostin expression levels by osteoblasts from adult rat bones submitted to chronic overload [59]. All these mechanisms are particularly important in periostin-induced response to environmental stress (i.e., mechanical loading and hypoxia) in order to maintain cell survival and functions in bone [54–56, 60, 61]. To increase periostin expression in response to an environmental stress may represent an adaptative cell strategy to maintain cell survival.

Cytokine regulation has not been, so far, studied in bone. However, interleukin (IL)-4 and IL-13, two anti-inflammatory cytokines that inhibit osteoblast proliferation but enhance their differentiation and matrix production, have been shown to induce periostin in an in vivo mouse model of fibrosis [17].

Besides its function in the formation of hydroxyapatite, inorganic phosphate can affect cell functions and gene expression. Indeed, elevation of phosphate as mineralization begins leads to a down-regulation of periostin gene expression in vitro, strengthening the association of periostin with the early stages of osteoblast differentiation and bone formation [28, 29].

All these data demonstrate that periostin is regulated by factors acting on preosteoblasts rather than on fully differentiated cells and underlines its importance in cell proliferation and early stages of osteoblast differentiation (Table 1).

Table 1 Factors involved in positive or negative regulation of periostin expression

Regulation	Positive	Negative
Transcription factors	Twist Runx-2 C-Fos/AP1	Wnt
Hormones, cytokines, and growth factors	PTH Estrogens TGF-β Activin BMP-2 Retinoic acid IL-4 IL-13 TNFα PDGF FGF Angiotensin	Leptin
Physicochemical factors	Mechanical stress Hypoxia	Phosphate Microgravity

Periostin promotes osteoblast adhesion, differentiation, and survival

In MC3T3 and primary rat osteoblasts, periostin inhibition abrogated cell proliferation and differentiation and reduced the expression of cbfa1 [9, 59]. These data confirm that periostin is expressed early in osteoblast and is involved in the differentiation process, possibly through integrin binding. Although the mechanisms involved in periostin/integrin binding in bone have not been analyzed in details, studies on cardiac and cancer cells have extensively reported that periostin can bind through its FAS-1 domains to the integrins αvβ3, αvβ5, and α6β4 and enhance cell proliferation and survival, migration, and metastasis [62–65]. However, osteoblasts and osteoclasts express different integrins, at least αvβ3 and αvβ5, which may mediate periostin signaling in bone. Periostin and αvβ3 are concomitantly expressed in human bone tissue, suggesting that periostin could recruit and attach osteoblasts to bone matrix. Downstream effectors of periostin binding to integrins include FAK, Rho/PI3-kinase, and Akt/PKB signaling pathways, which induce migration, proliferation, and matrix formation [18, 56, 62, 64–66]. In bone, periostin binding to αvβ3 may also activate the downstream FAK and Akt/PKB pathway, one of most potent prosurvival signaling pathways that has been demonstrated in UMR-106 osteoblast-like cells, to regulate cell migration and survival [26, 65, 67]. Finally, it is interesting to note that, as βig-H3, periostin contains dimers of Asp and Ile in its second and fourth FAS-1 domains, which have been shown to be implicated in βig-H3 adhesion to α3β1 expressing corneal epithelial cells [13].

However, transducing periostin signals is also achieved by nonintegrin receptors. Notch proteins are transmembrane receptors that affect osteoblast differentiation and play important roles in anti-cell death function under mechanical stress [52]. A reduced expression of Notch1 and its downstream effector Bcl-xl has been described in PDL and femur of periostin null mice compared to normal mice [56, 68, 69]. By binding to Notch, periostin up-regulates expression of Notch and Bcl-xl, leading to inhibition of cell death, especially in stress conditions.

Other pathways still have to be elucidated to further explain periostin action. Mechanical loading experiments on mouse bone have shown that periostin is up-regulated, while sclerostin, a potent antagonist of bone formation through inhibition of Wnt and BMP signaling, is down-regulated [46, 47, 67, 70]. Under mechanical stress, it is possible that periostin regulates sclerostin secretion by osteocytes through integrin signaling, to stimulate osteoblast activity and maintain bone integrity [67, 71]. The fact that periostin null mice had their bone biomechanical properties restored by injection of a sclerostin-blocking antibody further lends to support this hypothesis [67]. Alternatively, periostin may stimulate bone formation directly through Wnt/β-catenin signaling [36, 72].

In summary, these data show that periostin is a prosurvival protein, largely involved in cell response to environmental stress [61, 63, 68]. By binding to integrins or cell surface receptors, periostin can activate intracellular signaling pathways leading to inhibition of apoptosis through inactivation of caspases 3 and 9 and prosurvival signaling resulting in increased bone formation [12, 61, 66, 73].

Periostin is involved in ECM structure and organization

Periostin belongs to the matricellular proteins family, that is, proteins that regulate cell functions and cell–matrix interactions rather than contributing directly to the formation of structural elements [74, 75]. In addition to periostin, this family includes thrombospondin-1, osteonectin, osteopontin, tenascin-C, and tenascin-X. A common feature of these proteins is that several animal models in which a matricellular protein has been knocked out survive embryogenesis and show only mild phenotypes at birth, consistent with their partial contribution to structural integrity. However, these proteins still play a critical role in collagen assembly, particularly during wound healing. Interestingly and unlike many other matricellular proteins, periostin is also present in adult animal in collagen-rich tissues such as PDL and bone, suggesting a key role in connective tissue homeostasis.

Many studies have shown that periostin could bind to matrix proteins, thanks to its four central FAS-1 domains characteristic of the FAS-1 family of adhesion proteins and its N-term EMI-domain, which could be involved in protein–protein interactions [76]. However, the fact that periostin presents variants that are hardly secreted, as described for intact periostin, or showing a nuclear localization sequence, strongly suggests that besides its involvement in cell survival and ECM organization through binding to cell surface receptors and ECM proteins, periostin may also have intracellular functions [20].

Periostin binds to type I collagen and fibronectin and is a key regulator of collagen cross-linking

Periostin is strongly expressed in collagen-rich fibrous connective tissues subjected to constant mechanical stress in vivo, suggesting its involvement in their structure and integrity. Periostin colocalizes with collagen Iα1 chain in the ECM of mouse periosteum and binds collagen Iα1 through its N-term EMI domains [17, 45, 77]. Periosteum from periostin null mice exhibited aberrant collagen fibrillogenesis with an alteration in fibrils diameter and collagen cross-linking as observed in the skin, tendons, and heart [16, 18, 67, 77]. Indeed, the collagen content of femur was not affected, but the concentration of the enzymatic cross-links pyridinoline and deoxypyridinoline was decreased in the periostin−/− whole femur when compared to wild-type mice. We recently reported that not only the enzymatic cross-links but also the immature precursors DHLNL and HLNL were decreased in periostin-deficient mice, and their levels correlated with bone strength (Gineyts et al., abstract ASBMR 2011).

The covalent cross-linking of collagen is an essential step in its fibrillogenesis, which is catalyzed by lysyl oxidase (LOX). Calvarial osteoblast cells from periostin null mice show a decreased in LOX expression [78]. LOX is synthesized in an inactive form and activated by cleavage of its propeptide by BMP-1, which colocalizes with periostin inside the cell [78]. Using intact and domain-deletions forms of periostin and BMP-1, the same group demonstrated that the four tandem repeats of the FAS-1 domains of periostin directly interacted with the metalloproteinase domain of BMP-1 [78]. Similar experiments showed that cellular fibronectin interacts with BMP-1 and the N-terminal EMI domain of periostin, and that endogenous BMP-1 and fibronectin coimmunoprecipitate in cell culture models [16, 17, 79]. These results indicate that inside the cell, periostin interacts with BMP-1 to increase its deposition in the fibronectin matrix in close proximity of pro-LOX. BMP-1 then induces the proteolytic activation of pro-LOX, and promotes collagen cross-linking as assessed by the measurement of pyridinoline and deoxypyridinoline content in collagen [20, 78] (Fig. 2). These data demonstrate that intracellular periostin acts as a scaffold that increases the deposition of BMP-1 into the fibronectin matrix, to activate pro-LOX and promote collagen cross-linking [20]. Corroborating these data, overexpression of periostin in rats increased bone formation and bone mass, but also cardiac tissue viscosity, a measure of collagen cross-linking [24]. Additionally, periostin has been shown to bind to collagen type V [17]. Because BMP-1 is involved in the activation—via proteolytic maturation—of other extracellular proteins present in bone including different types of collagens, laminin 5, or probiglycan, these findings suggest that periostin may have a wider role in matrix organization [78].

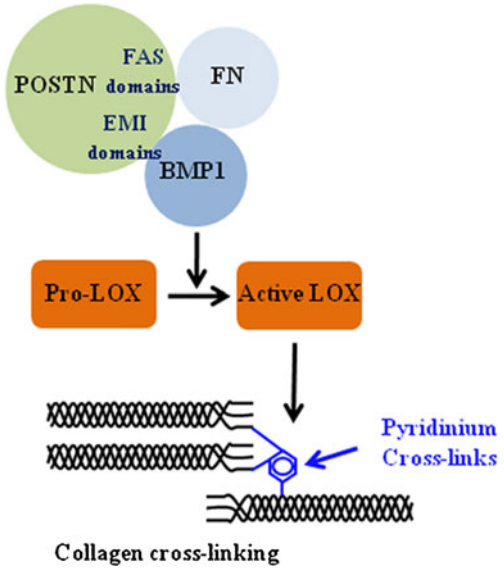

Fig. 2 Periostin is a key regulator of collagen cross-linking. Periostin (POSTN) binds fibronectin (FN) through its EMI domain and to BMP-1 through its FAS-1 domain, promoting the deposition of BMP-1 into the matrix. BMP-1 activates LOX precursor (pro-LOX) to mature active form (LOX). LOX is responsible for the synthesis of pyridinium cross-links, further linking collagen fibers

Overall, these data imply that, by its involvement in collagen fibril assembly and cross-linking, periostin is essential for proper collagen folding and maintenance of mechanical properties of collagen-rich tissues like bones (Table 2).

Periostin binds tenascin-C

Expressed in bone and myocardium and inducible by mechanical stimulation, tenascin-C forms a disulfide-linked hexamer called "hexabrachion" [16, 17, 80]. Periostin null mice showed an inhibition of the deposition of tenascin-C and a disorganization of the ECM at the lower tibia similar to the phenotype observed in the periostitis in tenascin-C null mice [16]. Periostin and tenascin-C present similarities in their expression profiles and are both induced by mechanical stress, implying that these two proteins are essential in the extracellular architecture of periosteum [75, 81]. Indeed, coimmunoprecipitation assays demonstrated that tenascin interacts with the FAS-1 domains of periostin lacking its C-terminal domain but not with intact periostin, suggesting that the C-terminal domain prevents aggregation of periostin with tenascin in the endoplasmic reticulum [16, 17]. Secreted as a cleaved form in the periosteum, periostin may support the incorporation of tenascin-C hexabrachions in the ECM by binding to tenascin and other proteins like fibronectin. This increases bifurcations of the matrix fibrils to support the meshwork architecture of the ECM [16, 20]. However in situ proximity ligation assays showed that both periostin and tenascin colocalize in the periphery or inside the cell, indicating a possible interaction of both proteins intracellularly or in the matricellular space. As suggested by Kudo [20] in his recent review, it is conceivable that periostin anchors tenascin in the intracellular network with fibronectin and BMP-1 to further induce collagen cross-linking.

Additionally, periostin also possesses a heparin-binding domain at its C-terminal end creating a potential binding site for glycoproteins, glycosaminoglycans, and proteoglycans [15]. Finally, periostin can also form disulfide-bonded dimers through its EMI domains [16, 17] (Table 2).

All these data clearly demonstrate that periostin may support intracellular and extracellular functions, with cellular or secreted isoforms that are differentially expressed [20]. By binding to endogenous fibronectin, tenascin, and BMP-1, cellular periostin is a key element in collagen cross-linking [78]. However, all these proteins are also expressed into the ECM, suggesting that periostin may be involved in extracellular collagen fibrillogenesis and meshwork architecture to support mechanical strength in periosteum. Periostin associates with other matricellular proteins such as Connective tissue growth factor, Cystein rich protein, and Nephroblastoma overexpressed gene (CCN) family members and thrombospondin known to be involved in some aspect of osteogenesis or chondrogenesis [20]. Furthermore, periostin has recently been shown to colocalize with laminin and fibronectin at the basement membrane in hair follicles, corroborating its central role in ECM organization [82].

Table 2 Periostin domains involved in interaction with ECM proteins

Periostin domain	Functions	ECM protein
N-terminal EMI domains	Protein interactions and protein multimerization	Collagen I Fibronectin Periostin
Fas-1 domains with Gla residues	Cell adhesion and protein interactions	BMP-1 Tenascin Calcium Integrins
C-terminal end	Potential binding site for glycoproteins	Heparin

Periostin is involved in bone strength and response to mechanical stress

First studies on periostin have highlighted its preferential expression in periosteum [1, 3]. Even if small changes in periosteal apposition occur throughout life, a growing

number of evidence that periosteum is a major contributor to bone strength are arising. Due to its external localization on bone, its high vascularity and support for tendons and muscle attachment, the periosteum may be more sensitive to mechanical and hormonal stimuli and largely involved in bone strength maintenance. Mechanical properties of long bones are more profoundly affected by modifications of the periosteal surface than the endocortical compartment [83, 84]. Indeed, periosteal osteoblasts show greater mechanosensivity and responsiveness to osteogenic compounds than endocortical ones. Mechanical stimulation induces an increase in bone strength by targeting new bone formation to the periosteum [85]. The preferential expression of periostin in periosteum suggests that it may be involved in bone microarchitecture and bone strength. Loss and gain of functions studies have clarified its importance in bone strength.

Periostin, bone mass, and microarchitecture

Periostin null mice are viable in utero and appear normal at birth, suggesting that periostin is not essential for in utero survival but may be rather required for events occurring later [2]. Indeed, some embryos died before weaning (14%), whereas some showed a decline in growth rate. Growth retardation was detectable 3 to 4 weeks after birth, with periostin−/− adult mice consistently smaller than their +/− and +/+ littermates. Their bones were undersized, cartilaginous growth plates were disrupted, trabecular network was restricted, and dwarfism persisted throughout life. Loss of periostin resulted in altered cancellous and cortical bone microarchitecture and lower bone mineral density [2, 67]. These defects in cortical microarchitecture were associated with reduced bone strength and turnover, without any significant differences in osteoblast and osteoclast number per bone surface [67]. Periostin+/− mice exhibited intermediate biomechanical characteristics, corroborating previous data in skin [77]. By contrast, bone-forming indices were unchanged at the endocortical surfaces in periostin null mice compared to normal littermates, in accordance with the absence of periostin expression in endosteum [3]. Periostin null mice appear to have normal developing teeth and periodontium at birth, but at 3 months, they show alveolar bone destruction, external root resorption, and incisor enamel defects, leading to disorganized enamel and dentin and increased tooth wear [2, 60, 86]. By contrast, overexpression of periostin in femur of 6-week-old rats increased bone formation and bone mass at least by increasing osteoblast activity [24].

All these data show that loss of periostin results in loss of integrity of bone and PDL matrix and suggest that periostin is a key regulator of cortical and cancellous bone microarchitecture and bone strength.

Periostin and response to mechanical stress

Physical activity has a positive effect on skeletal growth and development. Mechanical forces stimulate bone formation and suppress bone resorption, leading to an overall increase in bone mass. Cells from bone and PDL respond to stimulation by activation of mechanosensory signaling systems, cytoskeletal changes, and ECM architecture reorganization to withstand these loads without damage [87]. In periostin+/+ mice, axial compression and moderate physical activity significantly improve bone mineral density, trabecular and cortical microarchitecture, and biomechanical properties of long bones. Compression also increases bone formation, particularly in the periosteum. These stimulating effects are correlated with an elevated expression of periostin in the periosteum, particularly within the regions exhibiting the highest strain, that is, the proximal tibial midshaft. Periostin+/− mice showed a similar response to axial compression, but they were less affected by physical activity. By contrast, periostin−/− littermates responded neither to axial compression nor to exercise and showed a disorganized collagen matrix [67]. Furthermore, elevated expression of periostin mRNA in bone after axial compression in periostin+/+ mice was associated with an inhibition of sclerostin mRNA expression by osteocytes [67, 70]. By contrast, periostin−/− mice showed a higher baseline expression of sclerostin than periostin+/+, which remained unchanged following axial compression. However, their bone architecture and response to axial compression were restored by injection of a sclerostin-blocking antibody, suggesting that sclerostin was, in part, responsible for the lower bone mass of periostin-deficient mice [67]. As described earlier, periostin may down-regulate sclerostin secretion by osteocytes through integrin signaling, to maintain bone structure in stress condition. However, this does not exclude a direct action of periostin via Wnt/β-catenin. Furthermore, recent data have shown a reduced collagen cross-linking in tendons of periostin null mice [7]. As tendons are involved in the transmission of mechanical stress to bone, it is possible that alteration of tendons disrupts the mechanical transduction of strain resulting from compression or physical activity and, thus, is involved, at least in part, in the alteration of the bone response to mechanical stress in periostin null mice.

In teeth, PDL is involved in the transmission of physical forces resulting from mastication. Under mechanical strain, periostin mRNA and protein expression are increased in rodent and human PDL fibroblasts, compared to nonstimulated cells [33, 60, 88]. Furthermore, periostin overexpression was preceded by an up-regulation of TGF-β, a known activator of periostin expression, whereas periostin response to stress was abrogated by TGF-β neutralizing antibodies [33, 60, 88].

Similarly, inhibition of FAK/src pathway, involved in TGF-β activation, reduced nuclear translocation of twist and periostin expression in PDL fibroblasts, but also suppressed 3D collagen gel contraction induced by TGF-β [33]. Additionally, removal of occlusal forces caused a concomitant and transient inhibition of periostin and twist mRNA expression in PDL [32]. These data suggest that mechanical strain will activate latent TGF-β and twist-1 nuclear translocation, resulting in periostin activation. Finally, periostin null mice submitted to experimental tooth movement showed an increased PDL cells death associated with a reduced expression of Notch-1 and its downstream effector Bcl-xl, known to play an important role in cell survival under stress condition [67, 68]. These results suggest that periostin associates with Notch-1 to maintain its expression and subsequent signaling in order to enhance cell tolerance against the stress and protect them from death [68].

In conclusion, periostin knockout mice display a unique phenotype demonstrating that periostin is essential for bone and PDL development and functions in postnatal animals and highlight its crucial role in mechanotransduction [2, 60, 67]. In response to mechanical stress, periostin expression is up-regulated and activates different cellular pathways to support cell survival, to ensure a correct collagen fibrillogenesis and matrix organization to preserve tissue integrity and function. These data corroborate the results obtained with a rat model of chronic overload showing that, whereas variant 2 was induced at a constant high level all along the loading, variant 3 was expressed at the beginning of overload primarily in cellular periosteum, articular cartilage, osteoblasts, osteoclasts, and osteocytes in long bone. This rapid increase is concomitant to a transient increase in serum osteocalcin, indicative of adaptive bone formation, and suggests that periostin protein expression is associated with anabolic bone changes in response to loading. As the overload continued, immunoexpression of variant 3 decreased, paralleling with an increase in serum tartrate resistance acid phosphatase (TRAP5b)—a biological marker of bone resorption— thinning of the growth plate and reduced cortical thickness indicative of a pathological response to excess loading [27].

Periostin is a Gla-containing protein involved in mineralization

Localization of periostin in two mineralized tissues, bone and tooth, subjected to mechanical forces suggests a role in the regulation of mineralization. Indeed, the absence of periostin in genetically modified mice is associated with an early ossification, which will disturb the normal growth, impair tooth eruption, and result in dwarfism [2, 86]. In vitro, periostin mRNA expression was inversely related to the ability of cells to differentiate and mineralize, suggesting that periostin may be necessary to retard premature mineralization [45]. In fact, periostin is a vitamin K-dependent Gla-protein containing a consensus CRS, and many Glu-residues embedded within each of the four fasciclin-like domains of the protein. As γ-carboxylation is a posttranslational mechanism, it can be speculated that the different isoforms of periostin would be carboxylated [22]. Gla residues have the ability to bind divalent cations such as calcium and to incorporate them into hydroxyapatite crystals. In vitro, human bone marrow-derived stromal cells secrete periostin, part of which is carboxylated and abundantly localized in mineralized bone nodules, where osteoblasts are embedded [22]. Like osteocalcin and Matrix Gla-protein, two other bone Gla-proteins of bone, periostin may play a role in the regulation of ECM mineralization [89]. Osteocalcin and Matrix Gla-protein contain 3 and 5 Gla sites, respectively, whereas there are 28 potential sites in periostin and more than one functional CRS. This suggests that periostin could have a greater affinity for calcium, and a modulation in the degree of carboxylation may lead to regulation of periostin–hydroxyapatite binding [22]. By contrast, osteopontin and sialoprotein, two acidic and glutamate-rich proteins of bone, are rather responsible of nucleation of hydroxyapatite during the late maturation and mineralization phase of bone formation. Additionally, various degrees of carboxylation may, thus, also affect periostin interaction with ECM proteins and integrins. Clearly, the effect of γ-carboxylation of periostin on its properties requires further investigations.

All these data are in favor of a role of periostin in regulating premature and ectopic bone mineralization. Of interest, it has been shown that βig-h3 protein, a molecule-bearing fasciclin domains and highly homologous to periostin, inhibits bone nodule formation of osteoblasts in vitro [14].

Periostin is involved in bone repair

Periostin is preferentially expressed in periosteum, which plays a central role in bone formation during embryogenesis but also during fracture healing and bone repair. As a matricellular protein, periostin is largely expressed in embryos, declines during the course of skeletal growth, and is reexpressed in pathological conditions [75]. Periostin mRNA is among the most strongly up-regulated transcripts after tissue injury in the bone, heart, and muscle, suggesting a role in tissue regeneration [10, 11, 53, 90, 91]. Fracture healing occurs through direct or indirect repair, that is, intramembranous ossification or endochondral bone formation, respectively, that mimic early developmental processes. A tightly regulated cascade of molecular events is triggered soon after fracture with hematoma formation and inflammation, followed by the reparative phases, that is, angiogenesis, cartilage formation, and bone remodeling. In experimental rodent

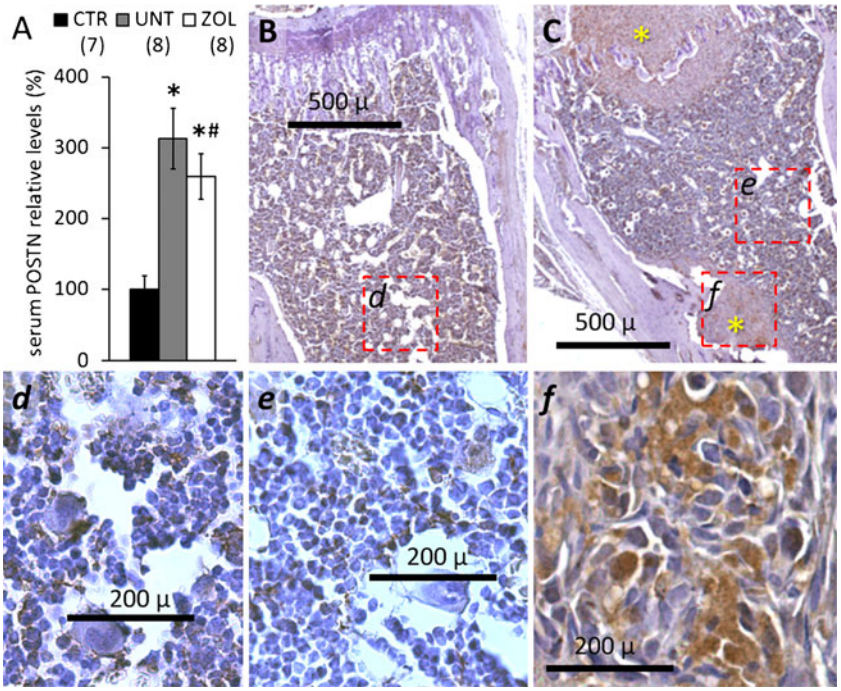

Fig. 3 Modulation of periostin expression levels in control animals (CTR) and vehicle (UNT)- and zoledronic acid (ZOL)-treated metastatic mice on day 32 after tumor cell inoculation. **a** Relative changes of serum periostin levels in control mice (n=7) and vehicle- (n=8) and zoledronic acid-treated (n=8) metastatic mice. Values are the mean (±SD) relative change compared to control group. *p<0.05 vs. CTR; #p<0.05 vs. UNT. **b** Representative immunostaining of periostin in hind limbs from control mice and **c** mice bearing bone metastases (asterisks). **d** Higher magnification of the marrow immunostaining image depicted in **b**. **e** Higher magnification of a field containing marrow not affected by metastatic tissue shown in **c**. **f** Higher magnification of metastatic tissue depicted in **c**. From Contie et al. [97]

models of fractures on long bone, it was shown that on day 3 postfracture, periostin mRNA was up-regulated in proliferating preosteoblastic and undifferentiated mesenchymal cells of the soft callus compared to unfractured bone. Its expression peaked at day 7 in the hard callus and decreased thereafter: it could still be detected in periosteal osteoblasts by day 14, when the hard callus has been replaced by woven bone [11, 24]. These results suggest that periostin is involved in early events of fracture repair, during the recruitment of progenitors into the callus and the early stages of osteoblast differentiation and bone formation. Similar expression of periostin has been demonstrated in the remodeling events occurring after vascular remodeling and infarct where periostin may induce reentry into the cell cycle leading to proliferation [66].

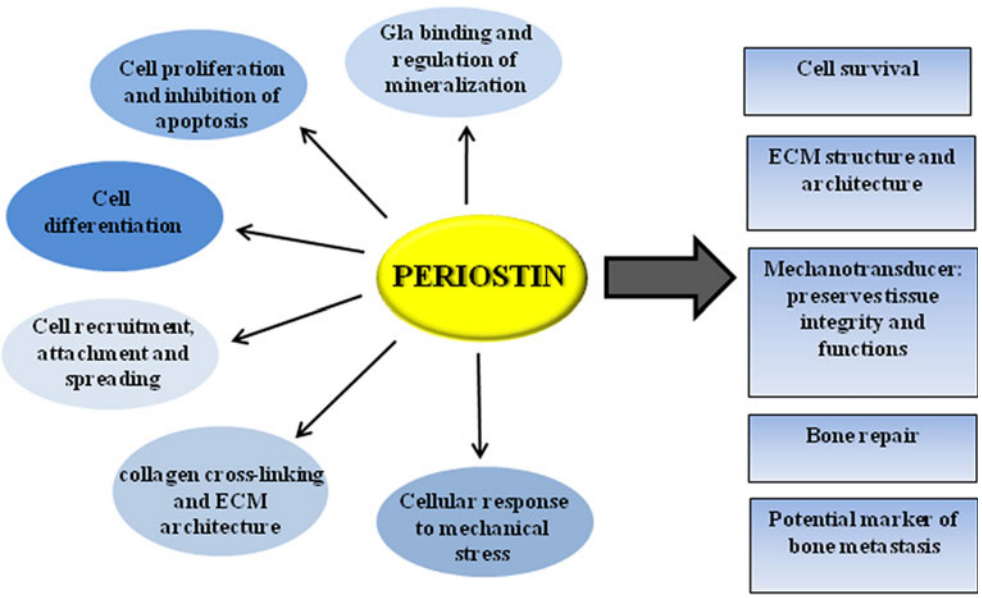

Fig. 4 Putative functions of periostin in bone. Periostin is involved in several cellular and extracellular functions resulting in stimulation of bone formation during development, response to mechanical stress, or bone fracture. Overexpressed in tumors, periostin may be a potential marker of bone metastasis

Data on systemic circulating levels of periostin are scarce. Using a mouse-specific enzyme-linked immunosorbent assay that we recently developed in our laboratory and recognizing all periostin isoforms, we have shown that serum levels of periostin decrease with skeletal growth and stabilize as adult age has been reached. Periostin levels do not correlate with bone formation or bone resorption markers in growth-stabilized animals and are not modulated by the bisphosphonate zoledronic acid. These data suggest that periostin does not reflect bone remodeling but, rather, ontogenic ossification processes [92]. Periostin, with its preferential location to periosteum, may play an active role in fracture repair, especially in the early events of bone regeneration.

Finally, it is of interest to report the overexpression of periostin in fibrous dysplasia, a bone disorder in which fibro-osseous tissue develops in place of normal bone, leading to bone fragility [26]. This is supportive of the importance of periostin in the maturation of collagen and ECM proteins. Furthermore, periostin may be a potential target in a therapy to treat ectopic fibro-osseous tissue formation in fibrous dysplasia and possibly joint diseases such as rheumatoid arthritis and osteoarthritis.

Periostin and bone metastasis

Cancer metastasis to the bone site represents a final stage of tumor progression. Periostin stimulates metastatic growth by promoting cell survival, invasion, and angiogenesis. Overexpression of periostin has been described in the stroma of many cancers and is associated with metastasis and poor prognosis in various cancers [93–95]. However, no data were reported on the expression of periostin in bone metastases, which are a frequent complication of solid tumor cancers, especially from prostate and breast origin [96]. Recently, in a mouse model of human breast cancer bone metastases using specific mRNA probes, we showed an overexpression of periostin in the stroma surrounding the bone metastases (Fig. 3c, f), compared to healthy bone (Fig. 3b, d, e), and an elevated circulating levels of periostin (Fig. 3a) [97]. Similarly, increased levels of circulating periostin were reported in women with breast cancer and bone metastases [98]. Moreover, a strong expression of periostin has been shown in the stroma of prostate cancer in patients with bone metastases [99, 100]. Markers available to assess bone metastases are associated with the process of bone formation and resorption. They cannot detect changes before the metastatic bone has proliferated and caused enough disruption of the balance between formation and resorption. Therefore, periostin could be an earlier marker of bone metastasis reflecting the change in bone marrow stroma under the influence of metastasis, a hypothesis that would need to be confirmed by longitudinal studies.

Conclusions

Periostin is a matricellular protein playing a fundamental role as an anabolic factor in bone and tooth tissue development and repair. By binding to cell surface receptors, it can modulate cell adhesion, proliferation, and differentiation, as well as cell–matrix interaction. Its involvement in collagen folding is crucial for matrix assembly and, therefore, bone strength (Fig. 4). The different periostin isoforms may contribute to a broad range of biological functions in both normal and pathological tissues. Thus, it will be interesting to analyze their differential expression according to time and site of bone formation. Periostin is a new Gla-protein identified in bone whose role in regulating mineralization has to be clarified. It is interesting to notice that βig-H3 protein, another FAS-1 protein rich in glutamate residues, inducible by TGF-β and able to bind similar integrins, is expressed in bone [14]. Finally, periostin possible involvement in connective tissue diseases is under scrutiny, as these disease commonly involve collagen, fibrillin, and elastin disorders, and periostin null mice show a phenotype similar to Marfan syndrome caused by a defect in fibrillin synthesis. The finding that periostin influences metastatic potential of tumors including breast and prostate tumors raises the possibility that it could be used as a molecular target in anti-metastasis therapy. Further studies are necessary to understand the regulation of periostin isoform expression and their biological activities in bone. The development of specific and sensitive assay for serum measurement will also be of crucial importance to investigate the potential of periostin as a biomarker of periosteal activity, bone quality, and/or bone metastases.

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