Osteopontin (OPN), a secreted phosphoprotein, is a member of the small integrin-binding ligand N-linked glycoprotein (SIBLING) family of cell matrix proteins and participates in many biological activities. Studies have shown that OPN plays a role in bone metabolism and homeostasis. OPN not only is an important factor in neuron-mediated and endocrine-regulated bone mass, but also is involved in biological activities such as proliferation, migration, and adhesion of several bone-related cells, including bone marrow mesenchymal stem cells, hematopoietic stem cells, osteoclasts, and osteoblasts. OPN has been demonstrated to be closely related to the occurrence and development of many bone-related diseases, such as osteoporosis, rheumatoid arthritis, and osteosarcoma. As expected, the functions of OPN in the bone have become a research hotspot. In this article, we try to decipher the mechanism of OPN-regulated bone metabolism and bone diseases.
Background

Osteopontin (OPN) is a transformation-associated phosphoprotein which was first reported by Senger in 1979 and named in 1985 by Franzén. OPN belongs to the small integrin-binding ligand N-linked glycoprotein (SIBLING) family [1] encoded by the SPP1 gene, which is mapped to the long arm of chromosome 4 [2] in a series array. In the bone marrow, OPN, accounts for about 2% of non-collagen bone and is mainly secreted by osteoblasts [3,4]. It can also be synthesized by osteogenesis progenitor cells, bone cells, and other hematopoietic cells in the bone marrow. OPN molecule is a multifunctional soluble extracellular matrix-associated glycoprotein [5–7] and has a specific arginine-glycine-aspartate (RGD) sequence [8], thus, it can be recognized, bound, and expressed by the corresponding integrin on the cell surface, making it important for cell adhesion and migration. OPN is secreted by cells undergoing complex translation and modifications, such as phosphorylation and glycosylation, to aid their biological activities and functions. Integrins (αvβ1 [9], αvβ3 [10], αvβ5 [9], αvβ6 [11], α4β1 [12], α4β7 [13], α5β1 [14], and α6β1 [15]) and CD44 [16] are the 2 major cell membrane surface receptors for OPN, which are highly correlated with many physiological and pathological processes such as biomineralization, cell-mediated immunity, inflammation, fibrosis, cell survival, tumorigenesis, and metastasis [17]. Recent studies have shown that OPN plays a role in neuron-mediated and endocrine-regulated bone mass. Also, OPN has been demonstrated to be closely related to the occurrence and development of many bone-related diseases such as osteoporosis, rheumatoid arthritis, and osteosarcoma. Osteoporosis caused by bone metabolism disorders is the most common bone disease in humans. Rheumatoid arthritis is a bone-related autoimmune disease that causes irreversible damage to the joints. Osteosarcoma is a common malignant tumor that occurs in bone tissue, which often causes lung metastasis and has a poor prognosis. A number of studies have indicated that abnormal expression of OPN is involved in the development of these skeletal diseases. This article reviews the mechanisms of OPN that affects bone metabolism through binding to related receptors, and reviews the role of OPN in bone diseases, including osteoporosis, rheumatoid arthritis, and osteosarcoma.

OPN Regulates Bone Development and Maintenance of Bone Mass

OPN plays an important role in neuron-mediated and endocrine-regulated bone mass

The sympathetic nervous system regulates bone mass by changing local bone remodeling through β2-adrenergic receptor (β2AR) [18]. OPN is a key factor in the regulation of bone mass by sympathetic nerves. It was found that isoproterenol stimulation of sympathetic nerves increased the mRNA and protein levels of OPN in plasma. OPN+β2AR mice inhibit isoproterenol-induced bone loss by preventing reduction of osteoblastic activity and addition of osteoclast activity. At the cell level, intracellular OPN regulates the ability of β2AR to generate cAMP. Furthermore, OPN correspondingly regulates cAMP-responsive element binding (CREB) phosphorylation and intracellular associated transcriptional events. Taken together, OPN participates in the sympathetic nervous system to regulate bone mass through the β2AR/cAMP signaling system [19].

Maintenance of bone homeostasis requires not only signal transduction, but also maintenance of normal calcium and phosphorus regulation. This complex process is regulated by several mechanisms of endocrine hormones, including parathyroid hormone (PTH), Klotho [20,21], FGF23 [22], and active vitamin D. Studies show that PTH directly affects mineralization disorders and skeletal deformities in Klotho−/− mice by regulating OPN expression [23]. Full expression of OPN is essential for PTH anabolism, while OPN knockdown can block PTH-stimulated alkaline phosphatase (ALP) activity and OCN (osteocalcin) expression, as well as induction of mineralization. Confirmedly, PTH-induced intracellular signaling is based on its receptor, the PTH/PTH-related protein (PTHrP) receptor (PPR) [24]. In bone, the extracellular matrix protein OPN is a negative regulator of PPR signaling in bone formation. Increased expression of OPN in osteoblasts by PPR signaling [25]. All these results indicate that OPN is an important factor in PTH regulation.

OPN Promotes Mesenchymal Stem Cells (MSCs) Proliferation and Migration

In order to maintain bone homeostasis, mesenchymal stem cells (MSCs) need to be collected on bone resorption surface. MSCs induce expression and secretion of OPN which enhance proliferation and migration of MSCs under the condition of extracellular Ca2+ released from the bone resorption surfaces during osteoclast-mediated bone resorption [26]. OPN increases the expression of integrin β1 on the surface of MSCs [27], and activates the FAK/ERK signaling pathway by binding to integrin β1 to induce the directional migration of MSCs [28,29]. Furthermore, OPN promotes MSCs migration by reducing the lamin A/C expression, which are components of the nuclear envelope belonging to the A-type lamins (including lamin A and lamin C) [30], and it makes MSCs decrease nuclear stiffness via FAK/ERK signaling pathway [31].

OPN promotes the transplantation of hematopoietic stem cells(HSCs)

The expression of OPN is proved to be restricted to the endosteal bone surface and conducted to transmarrow migration
of hematopoietic stem cells (HSCs) toward the endosteal region [32], as the distribution of HSCs in OPN−/− mice is abnormal after HSCs transplantation. With aging, OPN expression in the bone marrow stroma is reduced, expose young HSCs to an OPN−/− niche leads to a drop-in transplantation, long-term augmented frequency of HSCs and the loss of stem cells polarity. Exposure of aged HSCs to thrombin-cleaved OPN can slow down aging of HSCs, resulting in increased transplantation, decreasing HSCs frequency, increasing stem cells polarity and restoring the balance between peripheral blood lymphocytes and myeloid cells [33]. When HSCs differentiate into primitive hematopoietic cells stage, the cells exhibit specific adhesion to OPN by integrin β1, and OPN negatively regulates primitive hematopoietic cells proliferation in vitro [32,34].

**Migration, adhesion, and activation of osteoclasts (OCs) in an OPN-dependent manner**

OCs are formed by the fusion of mononuclear macrophages which are differentiated from myeloid progenitor cells of HSCs. OPN-induced osteoclasts (OCs) migration and adhesion are achieved by a step-wise signal activation, which is dependent on the membrane receptor integrin αvβ3 by passing RhoA and Rac1 mediated PI3K/PKCα-PKCβ signaling, whereas integrin αvβ3-mediated PI3K/PKCα-PKCβ/RhoA-Rac1 axis signaling is responsible for pre-osteoclast and mature osteoclast migration. As differentiation progresses, the dependence on the PKCβ/Rac1 signaling pathway involved [35].

Other studies have found that OPN can also affect the adhesion and spread of OCs through PLCγ affecting the PKCα/RhoA-Rac1 signaling pathway. At the same time, OPN-activated PLCγ can release Ca2+ and increase free Ca2+ in the cytoplasm. Ca2+ signal makes NFATc1 dephosphorylation to induce conformational changes, exposing nuclear localization signals and leading to translocation of NFATc1 to the nucleus and modification of transcription to enhance OCs survival [36,37].

The activation of OCs is in an OPN-dependent manner. High-level expression of αvβ3 is showed on OCs surface during bone injury. OPN, as a downstream signaling molecule for RANKL/NF-κB receptor activation, binds to αvβ3 in order to mediate OCs bone resorption. Due to the loss of OPN, the bone resorption capacity induced by PTH, RANKL, and M-CSF is decreased, and OCs proliferation is also affected [38–40].

**OPN affects osteoblast (OBs) activities**

There are some contradictions about the effect of OPN on osteoblasts (OBs). OPN can stimulate OBs proliferation, calcification, and mediate bone metabolism caused by mechanical stress [41]. Apart from this, oligomeric OPN promotes the adhesion of MC3T3-E1/C4 osteoblastic cell [42], and OPN has a good orientation for cell binding coupled with higher binding ability of college [43]. Nevertheless, other studies have demonstrated that OPN inhibits the proliferation and differentiation of OBs and inhibits mineral deposition in bone [44,45]. In the experiments to determine the effect of OPN and its 2 derived peptides on OBs development and mineralization, it is found that OPN plays an important role in mineralization, but has no effect on OBs development [46]. The cause of the difference may be related to experimental environmental conditions, drug dosage, or modification of OPN translation, and differences in target cell surface receptor expression.

**The Role of OPN in Bone-Related Diseases**

**High expression of OPN is a risk factor for osteoporosis**

Osteoporosis is a systemic skeletal disorder characterized by systemic damage to bone mass and microstructure, leading to fragility fractures [46–48]. Previous studies have shown that OPN was involved in bone strength and bone remodeling. The role of OPN in the regulation of bone metabolism has been proposed in many animal studies. The relationship between OPN levels and osteoporosis is gaining more and more attention.

Animal studies have shown that OPN-deficient mice by oophorectomy are resistant to osteoporosis [49]. Clinical studies have found that serum levels of OPN, as biomarkers for early diagnosis of osteoporosis in postmenopausal women, is positively related to the severity of osteoporosis. Compared with women who have normal OPN levels, overexpression of OPN is less resistant to postmenopausal osteoporosis. Further studies have found that serum OPN levels have significantly positive correlation with postmenopausal age, while is negatively correlated with weight, height, and material density of hips joints [50–52].

The expression of OPN can be mediated by estrogen receptor-related receptor α (ERRα). In vitro experiments show that ERRα regulates OPN transcription by activating the promoter of OPN gene. In vivo experiments, the dysregulation of OPN transcription is found in ovariectomized rat model. These results indirectly suggest the relationship between ERRα, OPN, and osteoporosis [53].

Although OPN decline has been observed in ERRα KO mice, there are the opposite views of whether ERRα can promote or inhibit differentiation of OBs. This is partly similar to the effect of OPN on OBs mentioned. Additionally, studies have shown that the number of OCs and bone resorption in elderly ERRα KO mice are significantly reduced [54].

Interestingly, ERRα expression is decreased in femur-derived RNA within 1 week after ovariectomy in rats, but it returns to normal 8 weeks post-ovariectomy [55].
normal after 2 weeks [55]. If these data can be extrapolated to humans, ERRαx expression would then be impossible to decrease significantly during long-term estrogen deficiency, and postmenopausal women may also express ERRαs in bone [56] to result in elevated OPN. The biological activities of the cells and bone metabolism can be affected further by OPN.

Osteoporotic subjects are vulnerable to suffer from bone fracture. Postmenopausal women have higher OPN levels, which increases the incidence of osteoporosis fractures to some extent [57]. However, the lack of OPN can lead to a 30% reduction in fracture toughness, wherefore OPN plays an important role in preventing fracture crack growth and is essential for fracture toughness in bone quality [58,59].

**OPN is involved in rheumatoid arthritis by regulating the immune response**

Rheumatoid arthritis is a chronic, inflammatory, autoimmune disease that accumulates multiple systems and its irreversible destruction of joints and joint deformities are the main reasons of joint function loss and disability. T cells in local joint injury has been confirmed to involve in the process of rheumatoid arthritis.

The expression of OPN and interleukin (IL)-17 increase in synovial fluid of rheumatoid arthritis patients. IL-17 is a highly inflammatory cytokine that activates OCs and causes destruction of cartilage matrix. Currently, IL-17 is considered to be a major factor in the secretion of Th1 cells in the synovium of the joint. There is dynamic balance and mutual inhibition between Th1/Th2 cell subsets. Once this dynamic equilibrium is broken, the body will be disease due to the condition of Th1/Th2 drift state that either Th1 dominant or Th2 dominant [60,61].

OPN binds to the corresponding receptor on the surface of T cells to not only promote Th1 type cells differentiation and enhances cellular immunity, but also inhibit Th2 type cells and humoral immune function. The imbalance of Th1/Th2 cells and the levels of secreted cytokines trigger a series of events leading to chronic inflammation and bone and cartilage destruction [62]. These results manifest that OPN plays a crucial role in the pathogenesis and progression of rheumatoid arthritis.

Studies have shown that IL-17 promotes monocyte migration via the Syk/P13K/Akt signaling pathway [63]. Local overexpression of IL-17 stimulates expression of NF-κB receptor activator ligand (RANKL) and its receptor leading to RANKL/osteoprotegerin (OPG) imbalance which can promote OCs proliferative activity in synovial fluid and bone erosion to accelerate rheumatoid arthritis damage [64–66]. After inhibition of OPN expression, joint swelling, cartilage erosion and monocyte infiltration are significantly improved [67]. OPN affects the balance of Th1/Th2 cells and induces Th17 cells differentiation through T cells surface receptors, then both conditions can affect IL-17 levels resulting in promoting OCs activity through Syk/P13K/Akt signaling pathway and NF-κB signaling pathway.

**OPN covers the occurrence, development and metastasis of osteosarcoma**

OPN plays a role in the occurrence and development of osteosarcoma. OPN is a late marker of osteogenic differentiation. The expression levels of OPN in osteosarcoma cells are significantly lower than that of differentiated mature OBs. Decreased OPN levels impair the differentiation of MSCs into OBs [68–70]. This is consistent with the presence of poorly differentiated tumor cells. Further during osteosarcoma development, deletion of the p53 gene leads to an increase in Notch signaling resulting in upregulation of cyclin and osterix and inhibition of Runx2, then a decrease in Runx2 inhibits OPN expression [71].

However, an increase in the expression levels of OPN possibly indicates the distance metastasis of osteosarcoma cells. Animal experiments have shown that osteosarcoma cells secrete higher levels of OPN as the main chemical in the migration process and show a high tendency to lung metastasis [72,73]. In tumors, glucose transporters are overexpressed related to metastasis. OPN induces the expression of hypoxia-responsive glucose transporter 1 and glucose transporter 3, and enhances glucose uptake by integrin αvβ3 [74]. Another aspect, an increase in HIF-1α activity leads to upregulation of OPN in osteosarcoma under hypoxic condition, and HIF-1α activity is increased by P13K-AKT and MAPK signaling pathways. In addition, aberrant expression of microRNA (miRNA) is also involved in the development and metastasis of cancer, in which overexpression of miR-4262 inhibits OPN-mediated invasion, whereas consumption of miR-4262 increases OPN-mediated cells invasion in osteosarcoma cells [75–77]. Clinical trials have shown that OPN expression levels are higher in osteosarcoma patients, and are associated with vascular endothelial growth factor (VEGF) and COX-2 expression, but OPN expression does not provide predictive information about osteosarcoma patient outcomes [78,79]. The results of the aforementioned research revealed high expression of OPN can affect multiple mechanisms of tumor metastasis.

OPN is involved in multiple steps of distant metastasis and is associated with bone metastasis of other types of tumors [80]. Early studies have reported that breast cancer, lung cancer, prostate cancer [81,82], and nasopharyngeal carcinoma [83] have elevated OPN levels after bone metastases. A possible mechanism is that the combination of OPN and integrin promotes changes in malignant cells that are beneficial for metastasis [84]. OPN is expressed as an immunological checkpoint.
by tumor cells, inhibits T cells activation, and confers tumor immune tolerance to the host [85].

**Inhibition of OPN Affects Local Tissue Ectopic Calcification**

Ectopic calcification is abnormal calcification of soft tissue, especially in blood vessels, heart valves, and kidneys [86]. OPN is a molecule that is always co-localized with ectopic calcification. The expression of OPN is increased under conditions of injury and disease, and is closely related to calcification deposits found in many pathologies including atherosclerotic lesions [87,88], aortic stenosis [89], kidney stones [90,91], and tumors [92]. The role and mechanism of OPN in ectopic calcification is mainly through the study of vascular calcification and mainly included 2 aspects. One is combined with hydroxyapatite. Hydroxyapatite is an alkali-calcium phosphate that is highly associated with the substituted bioapatite found in bones and teeth, as well as the composition in many ectopic calcifications. *In vitro* research supports OPN is an effective inhibitor of hydroxyapatite crystal formation and growth [93–97]. The specific RGD sequence of OPN has an aspartate-rich calcium binding region. The combination of negatively charged glutamate and aspartate residues, a serine/threonine kinase substrate site, and a putative calcium binding motif confer OPN the ability to bind large amounts of Ca²⁺ (50 moles of calcium to 1 mole of bone) [86,98]. Another is that OPN mediates regulation of monocyte-derived cell uptake to inhibit calcification. OPN is produced by stromal cells or inflammatory cells of ectopic calcification sites, combined with bioapatite to initially physically inhibit crystal growth. The combination of OPN and bioapatite provides both recognition sites and/or concentration gradients for macrophages and giant cells leading to local accumulation and upregulation of carbonic anhydrase II (CAII). Interestingly, OPN also controls the CAII through cAMP mediated OCs activity. As a result, the proton efflux is increased and the local microenvironment is acidified [86,96]. Local acidification then causes the residual bioapatite to dissolve. *In vitro* and *in vivo* experiments have shown that the lack of OPN alone does not lead to vascular calcification, but it promotes calcification in mice with matrix Gla protein (MGP) deficiency, eventually leading to vascular stenosis and even vascular rupture. Therefore, it is found in the body that high expression of OPN around arterial calcification may induce an inhibitory effect on local ectopic calcification in *vivo* [99–101].

A large number of studies suggest that there may be a common pathophysiological mechanism between postmenopausal osteoporosis and arterial calcification [102,103]. The RANKL/RANK/OPG system is the most concerned link between osteoporosis and arterial calcification and is considered to be a common regulator of its pathological changes [104]. The one possible mechanism is that OPN deletion or anti-OPN monoclonal antibody inhibits RANKL-induced OC differentiation and bone resorption function, and its associated OC adhesion, migration and survival are weakened [105,106]. Another mechanism is that estrogen may be the key modulator of RANK-L-RANKL signaling in the development of both arterial calcification and osteoporosis. Estrogen mainly acts through ERα to inhibit RANKL-induced vascular cell BMP-2 expression and matrix Gla protein (MGP) expression and then slow down vascular lesion progression [107,108].

Ectopic calcification could also occur in tumor tissue. Tumor microcalcification as a recognized pathological calcification mode is considered to be an early diagnostic marker for breast tumors, but its formation pathway and role in cancer progression are controversial. OPN has also been shown to be associated with calcification in human breast cancer [109]. The increase in OPN mRNA is detected in an *in vitro* model of mammary mineralization, but the addition of exocrine OPN to osteogenic cocktail does not inhibit mineralization [110,111]. The reason that exogenous OPN has no effect in this mammary mineralization model may be due to the higher endogenous levels of ALP in the 4T1 cell line used in this experiment [110]. ALP is known to dephosphorylate OPN [95,112] to eliminate its inhibitory effects. However, studies have reported that the addition of the ALP inhibitor levamisole could completely prevent mineralization [113]. The level of ALP decreases as the malignant potential decreases, while the level of OPN increases [109]. These findings suggest that, similar to vascular calcification, calcium deposition within breast lesions appears to be due to an active, regulated process of deposition of minerals.

**Conclusions**

OPN acts as a secreted protein that affected to many aspects of bone metabolism. OPN participates in bone regulation through neurology, endocrine and immunity. At the cellular levels, OPN participates in the biological activities of stem cells, OCs and OBs through relevant signaling pathways to regulate bone metabolism (Table 1). OPN plays different roles in different pathways, promoting activities along one pathway and inhibiting activities along another pathway. High levels OPN are an important risk factor for OP, which positively regulate OCs and inhibit bone mineral deposition, and whether it is related to the expression of ERα in osteoporosis patients requires further research to prove. Lack of OPN can increase fracture sensitivity in patients with osteoporosis. In the pathological process of rheumatoid arthritis disease, OPN is a crucial cytokine, which participates in pathological processes such as inflammatory reaction, vasospasm formation and bone damage. OPN expression is upregulated during tumorigenesis and development, while a decrease indicates tumors metastasis. The lack
of OPN affects the ectopic calcification of local tissues, but its individual necessity in ectopic calcification needs further research. Vascular calcification after menopause and microcalcification of breast tumor are associated with OPN. However, OPN still has many details not clear on the mechanism of bone metabolism and bone-related diseases. The in-depth study of OPN provides new ideas and directions for interpreting the pathogenesis of diseases and provides new targets to therapy with important clinical significance and value.

Conflict of interest.
None.

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Table 1. Molecular mechanism of OPN participating in cell biological activities.

| Species            | Cell type    | Function                  | Mechanism of action                                      | Reference |
|--------------------|--------------|---------------------------|----------------------------------------------------------|-----------|
| C57BL/6 mice       | MSCs         | Proliferation             | Elevated extracellular Ca\(^{2+}\) increases OPN expression | [26]      |
|                    | C3H10T1/2    | Migration                 |                                                          |           |
| C57BL6/129 mice    | MSCs         | Expansion                 | Fra-2 regulates OPN expression                            | [114]     |
| Sprague-Dawley rats| MSCs         | Migration                 |                                                          |           |
| MC3T3-E1           | Adhesive     |                           | Tissue transglutaminase (TG2) mediated oligomerization    | [41]      |
| MC3T3-E1           | Response to HGF and PDGF |                     | Inactivates FAK through the induction of LWM-PTP           | [43]      |
| MC3T3-E1           | Proliferation |                           | NF-κB pathway                                             | [115]     |
| C57BL/6 mice       | Osteoclast   | Adhesion                  | PLC/PI3K/PKCα/RhoA-Rac1 signaling                          | [36]      |
| C57BL/6 mice       | Osteoclast precursors | Migration            | PI3K/PKCα-PKCδ/RhoA-Rac1 signaling                       | [35]      |
| Wistar rats/New Zealand white rabbits | Osteoclast | Survival                  | Calcium-nuclear factor of activated T cells (NFAT)‐dependent pathway | [37]      |
| C57BL/6 mice       | Osteoclast   | Activity                  | PI3K and MEK/ERK pathways                                 | [40]      |
| Raw266.7           | Proliferation |                           | NF-κB pathway                                             | [115]     |
| Human              | Immature dendritic cells (iDC) | OC-like multinucleated giant cells (MGCs) differentiation | SVVYGLR fragment integrin receptor α4β1 and/or α9β1 | [116]     |
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