

Received: 2019.08.04
Accepted: 2019.11.04
Published: 2020.01.30

Osteopontin in Bone Metabolism and Bone Diseases

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2
BCF 1,2
EF 1,2
EF 3
DG 1,2
AFG 1,2

Jinyan Si
Chaowei Wang
Denghui Zhang
Bo Wang
Weiwei Hou
Yi Zhou

1 Affiliated Hospital of Stomatology, Medical College, Zhejiang University, Hangzhou, Zhejiang, P.R. China
2 Key Laboratory of Oral Biomedical Research of Zhejiang Province, Zhejiang University, Hangzhou, Zhejiang, P.R. China
3 Shanxi Medical University, Taiyuan, Shanxi, P.R. China





Corresponding Author: Yi Zhou, e-mail: zyuthscsa@zju.edu.cn

Source of support: This work was supported by Zhejiang Provincial Natural Science Foundation of China (grant nos. LQ18H140002) and Zhejiang Provincial Natural Science Foundation of China (No. LY19H140003)

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.

MeSH Keywords: **Bone Diseases • Bone Diseases, Metabolic • Osteopontin**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/919159>

 3344  1  —  116



Background

Osteopontin (OPN) is a transformation-associated phosphoprotein which was first reported by Senger in 1979 and named in 1985 by Franzen. 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 ($\alpha\upsilon\beta 1$ [9], $\alpha\upsilon\beta 3$ [10], $\alpha\upsilon\beta 5$ [9], $\alpha\upsilon\beta 6$ [11], $\alpha 4\beta 1$ [12], $\alpha 4\beta 7$ [13], $\alpha 5\beta 1$ [14], and $\alpha 9\beta 1$ [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-regulate 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 $\beta 2$ -adrenergic receptor ($\beta 2AR$) [18]. OPN is a key factor in the regulation of bone mass by sympathetic nervous. It was found that isoproterenol

stimulation of sympathetic nervous increased the mRNA and protein levels of OPN in plasma. OPN^{-/-} 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 $\beta 2AR$ to generate cAMP. Furthermore, OPN correspondingly regulates cAMP-response element binding (CREB) phosphorylation and intracellular associated transcriptional events. Taken together, OPN participates in the sympathetic nervous system to regulate bone mass through the $\beta 2AR$ /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. Confirmed, 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 $\beta 1$ on the surface of MSCs [27], and activates the FAK/ERK signaling pathway by binding to integrin $\beta 1$ 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 increasing 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 $\beta 1$, 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 $\alpha \beta 3$ by passing RhoA and Rac1 mediated PI3K/PKC α -PKC δ signaling, whereas integrin $\alpha \beta 3$ -mediated PI3K/PKC α -PKC δ /RhoA-Rac1 axis signaling is responsible for pre-osteoclast and mature osteoclast migration. As differentiation progresses, the dependence on the PKC δ /Rac1 signaling pathway involved [35].

Other studies have found that OPN can also affect the adhesion and spread of OCs through PLC γ affecting the PKC α /RhoA-Rac1 signaling pathway. At the same time, OPN-activated PLC γ 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 $\alpha \beta 3$ is showed on OCs surface during bone injury. OPN, as a downstream signaling molecule for RANKL/NF- κ B receptor activation, binds to $\alpha \beta 3$ 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 hip joints [50–52].

The expression of OPN can be mediated by estrogen receptor-related receptor α (ERR α). *In vitro* experiments show that ERR α 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 ERR α , OPN, and osteoporosis [53].

Although OPN decline has been observed in ERR α KO mice, there are the opposite views of whether ERR α 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 ERR α KO mice are significantly reduced [54].

Interestingly, ERR α expression is decreased in femur-derived RNA within 1 week after ovariectomy in rats, but it returns to

normal after 2 weeks [55]. If these data can be extrapolated to humans, $ERR\alpha$ expression would then be impossible to decrease significantly during long-term estrogen deficiency, and postmenopausal women may also express $ERR\alpha$ 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 in 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/PI3K/Akt signaling pathway [63]. Local overexpression of IL-17 stimulates expression of NF- κ B 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/PI3K/Akt signaling pathway and NF- κ B 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 $\alpha v \beta 3$ [74]. Another aspect, an increase in HIF-1 α activity leads to upregulation of OPN in osteosarcoma under hypoxic condition, and HIF-1 α activity is increased by PI3K-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 cells 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 Ca^{2+} (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 $\alpha v \beta 3$ 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-RANKL signaling in the development of both arterial calcification and osteoporosis. Estrogen mainly acts through $\text{ER}\alpha$ 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 is referred 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 $\text{ERR}\alpha$ 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

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

Species	Cell type	Function	Mechanism of action	Reference
C57BL/6 mice	MSCs C3H10T1/2	Proliferation Migration	Elevated extracellular Ca ²⁺ increases OPN expression	[26]
C57BL6/129 mice	MSCs	Expansion	Fra-2 regulates OPN expression	[114]
Sprague-Dawley rats	MSCs	Migration	FAK/ERK signaling	[28,29]
	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 Differentiation	NF-κB pathway	[115]
C57BL/6 mice	Osteoclast	Adhesion Spreading	PLCγ/PKCα/RhoA-Rac1 signaling	[36]
C57BL/6 mice	Osteoclast precursors Preosteoclasts Mature osteoclasts	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]
	Raw264.7	Proliferation Differentiation	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]

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.

References:

- Chen Q, Shou P, Zhang L et al: An osteopontin-integrin interaction plays a critical role in directing adipogenesis and osteogenesis by mesenchymal stem cells. *Stem Cells*, 2014; 32(2): 327–37
- Kothari A, Arffa M, Chang V et al: Osteopontin – a master regulator of epithelial-mesenchymal transition. *J Clin Med*, 2016; 5(4): pii: E39
- Denhardt DT, Giachelli CM, Rittling SR: Role of osteopontin in cellular signaling and toxicant injury. *Ann Rev Pharmacol Toxicol*, 2001; 41(1): 723–49
- Mckee MD, Nanci A: Osteopontin: An interfacial extracellular matrix protein in mineralized tissues. *Connect Tissue Res*, 2009; 35(1–4): 197–205
- Mark MP, Butler WT, Prince CW et al: Developmental expression of 44-kDa bone phosphoprotein (osteopontin) and bone gamma-carboxyglutamic acid (Gla)-containing protein (osteocalcin) in calcifying tissues of rat. *Differentiation*, 2010; 37(2): 123–36
- Mark MP, Prince CW, Oosawa T et al: Immunohistochemical demonstration of a 44-KD phosphoprotein in developing rat bones. *J Histochem Cytochem*, 1987; 35(7): 707–15
- Swanson GJ, Nomura S, Hogan BLM: Distribution of expression of 2AR (osteopontin) in the embryonic mouse inner ear revealed by *in situ* hybridisation. *Hear Res*, 1989; 41(2): 169–77
- Oldberg A, Franzen A, Heinegard D: Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an Arg-Gly-Asp cell-binding sequence. *Proc Natl Acad Sci USA*, 1986; 83(23): 8819–23
- Hu DD, Lin EC, Kovach NL et al: A biochemical characterization of the binding of osteopontin to integrins alpha v beta 1 and alpha v beta 5. *J Biol Chem*, 1995; 270(44): 26232–38

10. Liaw L, Skinner MP, Raines EW et al: The adhesive and migratory effects of osteopontin are mediated by distinct cell surface integrins. Role of alpha v beta 3 in smooth muscle cell migration to osteopontin *in vitro*. *J Clin Invest*, 1995; 95(2): 713–24
11. Yokosaki Y, Tanaka K, Higashikawa F et al: Distinct structural requirements for binding of the integrins $\alpha\beta 6$, $\alpha\beta 3$, $\alpha\beta 5$, $\alpha 5\beta 1$ and $\alpha 9\beta 1$ to osteopontin. *Matrix Biol*, 2005; 24(6): 418–27
12. Bayless KJ, Meininger GA, Scholtz JM, Davis GE: OPN is a ligand for the alpha4beta1 integrin. *J Cell Sci*, 1998; 111(Pt 9): 116574
13. Green PM, Ludbrook SB, Miller DD et al: Structural elements of the osteopontin SVVYGLR motif important for the interaction with $\alpha 4$ integrins. *FEBS Lett*, 2001; 503(1): 75–79
14. Barry ST, Ludbrook SB, Murrison E, Horgan CMT: A regulated interaction between $\alpha 5\beta 1$ integrin and osteopontin. *Biochem Biophys Res Commun*, 2000; 267(3): 764–69
15. Smith LL, Giachelli CM: Structural requirements for $\alpha 9\beta 1$ -mediated adhesion and migration to thrombin-cleaved osteopontin. *Exp Cell Res*, 1998; 242(1): 351–60
16. Naor D: CD44: Structure, function, and association with the malignant process. *Adv Cancer Res*, 1997; 71: 241–319
17. Farrokhi V, Chabot JR, Neubert H, Yang Z: Assessing the feasibility of neutralizing osteopontin with various therapeutic antibody modalities. *Sci Rep*, 2018; 8(1): 7781
18. Elefteriou F: Regulation of bone remodeling by the central and peripheral nervous system. *Arch Biochem Biophys*, 2008; 473(2): 231–36
19. Nagao M, Feinstein TN, Ezura Y et al: Sympathetic control of bone mass regulated by osteopontin. *Proc Natl Acad Sci USA*, 2011; 108(43): 17767
20. Komaba H, Kaludjerovic J, Hu DZ et al: Klotho expression in osteocytes regulates bone metabolism and controls bone formation. *Kidney Int*, 2017; 92(3): 599–611
21. Shun-Ai L, Masami W, Hiroshi Y et al: Immunohistochemical localization of klotho protein in brain, kidney, and reproductive organs of mice. *Cell Struct Funct*, 2004; 29(4): 91–99
22. Kuro-o M, Moe OW: FGF23- α Klotho as a paradigm for a kidney-bone network. *Bone*, 2017; 100: 4–18
23. Ihara H: Parathyroid hormone-induced bone resorption does not occur in the absence of osteopontin. *J Biol Chem*, 2001; 276(16): 13065–71
24. Morishita M, Ono N, Miyai K et al: Osteopontin deficiency enhances parathyroid hormone/parathyroid hormone related peptide receptor (PPR) signaling-induced alteration in tooth formation and odontoblastic morphology. *Tissue Cell*, 2011; 43(3): 196–200
25. Boulefour W, Bouet G, Granito RN et al: Blocking the expression of both bone sialoprotein (BSP) and osteopontin (OPN) impairs the anabolic action of PTH in mouse calvaria bone. *J Cell Physiol*, 2015; 230(3): 568–77
26. Lee MN, Hwang H-S, Oh S-H et al: Elevated extracellular calcium ions promote proliferation and migration of mesenchymal stem cells via increasing osteopontin expression. *Exp Mol Med*, 2018; 50: 142
27. Zou C, Song G, Luo Q et al: Mesenchymal stem cells require integrin $\beta 1$ for directed migration induced by osteopontin *in vitro*. *In Vitro Cellular & Developmental Biology – Animal*, 2010; 47(3): 241–50
28. Liu L, Luo Q, Sun J et al: Decreased nuclear stiffness via FAK-ERK1/2 signaling is necessary for osteopontin-promoted migration of bone marrow-derived mesenchymal stem cells. *Exp Cell Res*, 2017; 355(2): 172–81
29. Zou C, Luo Q, Qin J et al: Osteopontin promotes mesenchymal stem cell migration and lessens cell stiffness via integrin $\beta 1$, FAK, and ERK pathways. *Cell Biochem Biophys*, 2012; 65(3): 455–62
30. Genschel J, Schmidt HH-J: Mutations in the LMNA gene encoding lamin A/C. *Human Mut*, 2015; 16(6): 451–59
31. Chatakun P, Núñez-Toldrà R, Díaz López EJ et al: The effect of five proteins on stem cells used for osteoblast differentiation and proliferation: A current review of the literature. *Cell Mol Life Sci*, 2013; 71(1): 113–42
32. Nilsson SK, Johnston HM, Whitty GA et al: Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood*, 2005; 106(4): 1232–39
33. Guidi N, Sacma M, Standker L et al: Osteopontin attenuates aging associated phenotypes of hematopoietic stem cells. *EMBO J*, 2017; 36(7): 840–53
34. Haylock DN, Nilsson SK: Osteopontin: A bridge between bone and blood. *Br J Haematol*, 2010; 134(5): 467–74
35. Kim J-M, Kim MY, Lee K, Jeong D: Distinctive and selective route of PI3K/PKC α -PKC δ /RhoA-Rac1 signaling in osteoclastic cell migration. *Mol Cell Endocrinol*, 2016; 437(C): 261–67
36. Kim JM, Lee K, Jeong D: Selective regulation of osteoclast adhesion and spreading by PLC γ /PKC α -PKC δ /RhoA-Rac1 signaling. *BMB Rep*, 2018; 51(5): 230–35
37. Tanabe N, Wheal BD, Kwon J et al: Osteopontin signals through calcium and nuclear factor of activated T cells (NFAT) in osteoclasts. *J Biol Chem*, 2011; 286(46): 39871
38. Chellaiah MA, Hruska KA: The Integrin alpha(v)beta(3) and CD44 regulate the actions of osteopontin on osteoclast motility. *Calcif Tissue Int*, 2003; 72(3): 197–205
39. Sun Y, Yao Y, Ding C-Z: A combination of sinomenine and methotrexate reduces joint damage of collagen induced arthritis in rats by modulating osteoclast-related cytokines. *Int Immunopharmacol*, 2014; 18(1): 135–41
40. Walker CG, Dangaria S, Ito Y et al: Osteopontin is required for unloading-induced osteoclast recruitment and modulation of RANKL expression during tooth drift-associated bone remodeling, but not for super-eruption. *Bone*, 2010; 47(6): 1020–29
41. Forsprecher J, Wang Z, Goldberg HA, Kaartinen MT: Transglutaminase-mediated oligomerization promotes osteoblast adhesive properties of osteopontin and bone sialoprotein. *Cell Adh Migr*, 2011; 5(1): 65–72
42. Bernards MT, Qin C, Ratner BD, Jiang S: Adhesion of MC3T3-1 cells to bone sialoprotein and bone osteopontin specifically bound to collagen I. *J Biomed Mater Res A*, 2010; 86A(3): 779–87
43. Kusuyama J, Bandow K, Ohnishi T et al: Osteopontin inhibits osteoblast responsiveness through the downregulation of focal adhesion kinase mediated by the induction of low molecular weight-protein tyrosine phosphatase. *Mol Biol Cell*, 2017; 28(10): 1326–36
44. Holm E, Gleberzon Jared S, Liao Y et al: Osteopontin mediates mineralization and not osteogenic cell development *in vitro*. *Biochem J*, 2014; 464(3): 355–64
45. Huang W, Carlsen B, Rudkin G et al: Osteopontin is a negative regulator of proliferation and differentiation in MC3T3-E1 pre-osteoblastic cells. *Bone*, 2004; 34(5): 799–808
46. Christiansen C, Riis P: [Consensus development conference: Prophylaxis and treatment of osteoporosis.] *Ugeskr Laeger*, 1991; 149(48): 3270–72 [in Danish]
47. Compston JE, McClung MR, Leslie WD: Osteoporosis. *Lancet*, 2019; 393(10169): 364–76
48. Rachner TD, Khosla S, Hofbauer LC: Osteoporosis: Now and the future. *Lancet*, 2011; 377(9773): 1276–87
49. Yoshitake H, Rittling SR, Denhardt DT, Noda M: Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. *Proc Natl Acad Sci USA*, 1999; 96(14): 8156–60
50. Chang IC, Chiang TI, Yeh KT et al: Increased serum osteopontin is a risk factor for osteoporosis in menopausal women. *Osteoporos Int*, 2010; 21(8): 1401–9
51. Chiang TI, Chang IC, Lee HS et al: Osteopontin regulates anabolic effect in human menopausal osteoporosis with intermittent parathyroid hormone treatment. *Osteoporos Int*, 2010; 22(2): 577–85
52. Fodor D, Bondor C, Albu A et al: The value of osteopontin in the assessment of bone mineral density status in postmenopausal women. *J Investig Med*, 2013; 61(1): 15–21
53. Zirngibl RA, Chan JSM, Aubin JE: Estrogen receptor-related receptor (ERR) regulates osteopontin expression through a non-canonical ERR response element in a cell context-dependent manner. *J Mol Endocrinol*, 2008; 40(2): 61–73
54. Wei W, Xueqian W, Marie Y et al: PGC1beta mediates PPARgamma activation of osteoclastogenesis and rosiglitazone-induced bone loss. *Cell Metab*, 2010; 11(6): 503–16
55. Bonnelye E, Kung V, Laplace C et al: Estrogen receptor-related receptor α impinges on the estrogen axis in bone: Potential function in osteoporosis. *Endocrinology*, 2002; 143(9): 3658–70
56. Gallet M, Vanacker J-M: ERR receptors as potential targets in osteoporosis. *Trends Endocrinol Metab*, 2010; 21(10): 637–41
57. Hernandez CJ, Keaveny TM: A biomechanical perspective on bone quality. *Bone*, 2006; 39(6): 1173–81
58. Tanaka S, Narusawa K, Onishi H et al: Lower osteocalcin and osteopontin contents of the femoral head in hip fracture patients than osteoarthritis patients. *Osteoporos Int*, 2010; 22(2): 587–97

59. Thurner PJ, Chen CG, Ionova-Martin S et al: Osteopontin deficiency increases bone fragility but preserves bone mass. *Bone*, 2010; 46(6): 1564–73
60. Cañete JD, Martínez SE, Farrés J et al: Differential Th1/Th2 cytokine patterns in chronic arthritis: interferon gamma is highly expressed in synovium of rheumatoid arthritis compared with seronegative spondyloarthropathies. *Ann Rheum Dis*, 2000; 59(4): 263–68
61. Schulze-Koops H, Kalden JR: The balance of Th1/Th2 cytokines in rheumatoid arthritis. *Best Pract Res Clin Rheumatol*, 2001; 15(5): 677–91
62. Tsai C-H, Liu S-C, Wang Y-H et al: Osteopontin inhibition of miR-129-3p enhances IL-17 expression and monocyte migration in rheumatoid arthritis. *Biochim Biophys Acta*, 2017; 1861(2): 15–22
63. Ito H, Yamada H, Shibata TN: Dual role of interleukin-17 in pannus growth and osteoclastogenesis in rheumatoid arthritis. *Arthritis Res Ther*, 2011; 13(1): R14
64. Geusens P: The role of RANK ligand/osteoprotegerin in rheumatoid arthritis. *Ther Adv Musculoskelet Dis*, 2012; 4(4): 225–33
65. Honma M, Ikebuchi Y, Kariya Y, Suzuki H: Regulatory mechanisms of RANKL presentation to osteoclast precursors. *Curr Osteoporos Rep*, 2014; 12(1): 115–20
66. Honorati MC, Neri S, Cattini L, Facchini A: Interleukin-17, a regulator of angiogenic factor release by synovial fibroblasts. *Osteoarthritis Cartilage*, 2006; 14(4): 345–52
67. Qian J, Xu L, Sun X et al: Adiponectin aggravates bone erosion by promoting osteopontin production in synovial tissue of rheumatoid arthritis. *Arthritis Res Ther*, 2018; 20(1): 26
68. Luo X, Chen J, Song W-X et al: Osteogenic BMPs promote tumor growth of human osteosarcomas that harbor differentiation defects. *Lab Invest*, 2008; 88(12): 1264–77
69. Mackie PS, Fisher JL, Zhou H, Choong PFM: Bisphosphonates regulate cell growth and gene expression in the UMR 106-01 clonal rat osteosarcoma cell line. *Br J Cancer*, 2001; 84(7): 951–58
70. Tang N, Song W-X, Luo J et al: Osteosarcoma development and stem cell differentiation. *Clin Orthop Relat Res*, 2008; 466(9): 2114–30
71. Engin F, Bertin T, Ma O et al: Notch signaling contributes to the pathogenesis of human osteosarcomas. *Hum Mol Genet*, 2009; 18(8): 1464–70
72. Denhardt DT, Mistretta D, Chambers AF et al: Transcriptional regulation of osteopontin and the metastatic phenotype: Evidence for a Ras-activated enhancer in the human OPN promoter. *Clin Exp Metastasis*, 2003; 20(1): 77–84
73. Velupillai P, Sung CK, Tian Y et al: Polyoma virus-induced osteosarcomas in inbred strains of mice: host determinants of metastasis. *PLoS Pathogens*, 2010; 6(1): e1000733
74. Hsieh IS, Yang RS, Fu WM: Osteopontin upregulates the expression of glucose transporters in osteosarcoma cells. *PLoS One*, 2014; 9(10): e109550
75. Chang J, Yao M, Li Y et al: MicroRNAs for osteosarcoma in the mouse: A meta-analysis. *Oncotarget*, 2016; 7(51): 85650–74
76. Sasaki R, Osaki M, Okada F: MicroRNA-based diagnosis and treatment of metastatic human osteosarcoma. *Cancers*, 2019; 11(4): pii: E553
77. Song K, Liu N, Yang Y, Qiu X: Regulation of osteosarcoma cell invasion through osteopontin modification by miR-4262. *Tumor Biol*, 2015; 37(5): 6493–99
78. Liao Y, Li F, Hu X: Expression and clinical significance of OPN and COX-2 in osteosarcoma. *The Chinese-German Journal of Clinical Oncology*, 2007; 6(4): 378–82
79. Sulzbacher I, Birner P, Trieb K et al: Expression of osteopontin and vascular endothelial growth factor in benign and malignant bone tumors. *Virchows Arch*, 2002; 441(4): 345–49
80. Kruger TE, Miller AH, Godwin AK, Wang J: Bone sialoprotein and osteopontin in bone metastasis of osteotropic cancers. *Crit Rev Oncol Hematol*, 2014; 89(2): 330–41
81. D'Oronzo S, Brown J, Coleman R: The value of biomarkers in bone metastasis. *Eur J Cancer Care*, 2017; 26(6): e12725
82. Mountziou G, Dimopoulos M-A, Bamias A et al: Abnormal bone remodeling process is due to an imbalance in the receptor activator of nuclear factor- κ B ligand (RANKL)/osteoprotegerin (OPG) axis in patients with solid tumors metastatic to the skeleton. *Acta Oncol*, 2007; 46(2): 221–29
83. Hou X, Wu X, Huang P et al: Osteopontin is a useful predictor of bone metastasis and survival in patients with locally advanced nasopharyngeal carcinoma. *Int J Cancer*, 2015; 137(7): 1672–78
84. Schneider JG, Amend SR, Weilbaecher KN: Integrins and bone metastasis: Integrating tumor cell and stromal cell interactions. *Bone*, 2011; 48(1): 54–65
85. Klement JD, Paschall AV, Redd PS et al: An osteopontin/CD44 immune checkpoint controls CD8+ T cell activation and tumor immune evasion. *J Clin Invest*, 2018; 128(12): 5549–60
86. Steitz SA, Speer MY, McKee MD et al: Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol*, 2002; 161(6): 2035–46
87. Breyne J, Juthier F, Corseaux D et al: Atherosclerotic-like process in aortic stenosis: activation of the tissue factor-thrombin pathway and potential role through osteopontin alteration. *Atherosclerosis*, 2010; 213(2): 369–76
88. Giachelli CM, Bae N, Almeida M et al: Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. *J Clin Invest*, 1993; 92(4): 1686–96
89. O'Brien KD, Kuusisto J, Reichenbach DD et al: Osteopontin is expressed in human aortic valvular lesions. *Circulation*, 1995; 92(8): 2163–68
90. Vernon HJ, Christine O, Tzortzaki EG et al: Aprt/Opn double knockout mice: Osteopontin is a modifier of kidney stone disease severity. *Kidney Int*, 2005; 68(3): 938–47
91. Kohri K, Nomura S, Kitamura Y et al: Structure and expression of the mRNA encoding urinary stone protein (osteopontin). *J Biol Chem*, 1993; 268(20): 15180–84
92. Bellahcène A, Castronovo V: Expression of bone matrix proteins in human breast cancer: Potential roles in microcalcification formation and in the genesis of bone metastases. *Bull Cancer*, 1997; 84(1): 17–24
93. Wada T, McKee MD, Steitz S, Giachelli CM: Calcification of vascular smooth muscle cell cultures inhibition by osteopontin. *Circ Res*, 1999; 84(2): 166–78
94. Hunter GK, Kyle CL, Goldberg HA: Modulation of crystal formation by bone phosphoproteins: structural specificity of the osteopontin-mediated inhibition of hydroxyapatite formation. *Biochem J*, 1994; 300 (Pt 3)(3): 723–28
95. Boskey AL, Maresca M, Ullrich W et al: Osteopontin-hydroxyapatite interactions *in vitro*: Inhibition of hydroxyapatite formation and growth in a gelatin-gel. *Bone Miner*, 1993; 22(2): 147–59
96. Giachelli C, Steitz S: Osteopontin: A versatile regulator of inflammation and biomineralization. *Matrix Biol*, 2000; 19(7): 615–22
97. Jono S, Peinado C, Giachelli CM: Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *J Biol Chem*, 2000; 275(26): 20197–203
98. Chen Y, Bal BS, Gorski JP: Calcium and collagen binding properties of osteopontin, bone sialoprotein, and bone acidic glycoprotein-75 from bone. *J Biol Chem*, 1992; 267(34): 24871–78
99. Mei Y, Speer, McKee MD, Guldberg RE et al: Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: Evidence for osteopontin as an inducible inhibitor of vascular calcification *in vivo*. *J Exp Med*, 2002; 196(8): 1047–55
100. Paolo P, Grau JB, Field BC et al: Osteopontin controls endothelial cell migration *in vitro* and in excised human valvular tissue from patients with calcific aortic stenosis and controls. *J Cell Physiol*, 2011; 226(8): 2139–49
101. Lomashvili KA, Scott C, Hennigar RA et al: Phosphate-induced vascular calcification: Role of pyrophosphate and osteopontin. *J Am Soc Nephrol*, 2004; 15(6): 1392–401
102. Wada H, Hirano F, Kuroda T, Shiraki M: Breast arterial calcification and hypertension associated with vertebral fracture. *Geriatr Gerontol Int*, 2012; 12(2): 330–35
103. Jørgensen L, Joakimsen O, Mathiesen EB et al: Carotid plaque echogenicity and risk of nonvertebral fractures in women: A longitudinal population-based study. *Calcif Tissue Int*, 2006; 79(4): 207–13
104. D'Amelio P, Isaia G, Isaia GC: The osteoprotegerin/RANK/RANKL system: a bone key to vascular disease. *Expert Rev Cardiovasc Ther*, 2006; 4(6): 801–11
105. Du X, Huang X, Cui H et al: Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. *J Dent*, 2012; 40(6): 485–92
106. Zhao J, Gui S, Wang X: [Osteopontin expression of pulmonary artery in pulmonary atherosclerosis model rabbit.] *Acta Universitatis Medicinalis Anhui*, 2012 [in Chinese]
107. Osako MK, Nakagami HN, Shimizu H et al: Estrogen inhibits vascular calcification via vascular RANKL system: Common mechanism of osteoporosis and vascular calcification. *Circ Res*, 2010; 20(4): 466–75

108. Bourassa PA, Milos PM, Gaynor BJ et al: Estrogen reduces atherosclerotic lesion development in apolipoprotein E-deficient mice. *Proc Natl Acad Sci USA*, 1996; 93(19): 10022–27
109. Vidavsky N, Kunitake JA, Chiou AE et al: Studying biomineralization pathways in a 3D culture model of breast cancer microcalcifications. *Biomaterials*, 2018; 179: 71–82
110. Cox RF, Hernandez-Santana A, Ramdass S et al: Microcalcifications in breast cancer: Novel insights into the molecular mechanism and functional consequence of mammary mineralisation. *Br J Cancer*, 2012; 106(3): 525–37
111. Addison WN, Fereshteh A, Sorensen ES et al: Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity. *J Biol Chem*, 2007; 282(21): 15872–83
112. Gericke A, Qin C, Spevak L et al: Importance of phosphorylation for osteopontin regulation of biomineralization. *Calcif Tissue Int*, 2005; 77(1): 45–54
113. O'Grady S, Morgan MP: Microcalcifications in breast cancer: From pathophysiology to diagnosis and prognosis. *Biochim Biophys Acta*, 2018; 1869(2): 310–20
114. Luo Y, Grötsch B, Nicole Hannemann N et al: Fra-2 expression in osteoblasts regulates systemic inflammation and lung injury through osteopontin. *Mol Cell Biol*, 2018; 38(22): pii: e00022-18
115. Dong M, Yu X, Chen W et al: Osteopontin promotes bone destruction in periapical periodontitis by activating the NF-κB Pathway. *Cell Physiol Biochem*, 2018; 49(3): 884–98
116. Oh Y, Oh I, Morimoto J et al: Osteopontin has a crucial role in osteoclast-like multinucleated giant cell formation. *J Cell Biochem*, 2014; 115(3): 585–95