Current Osteoporosis Reports (2019) 17:97–104
https://doi.org/10.1007/s11914-019-00507-z

OSTEOCYTES (J KLEIN NULEND, SECTION EDITOR)

Pro-inflammatory Cytokines and Osteocytes

Miao Zhou[1] · Shuyi Li[1] · Janak L. Pathak[1]

Published online: 26 March 2019

Abstract

Purpose of Review An elevated level of pro-inflammatory cytokines in inflammatory conditions causes bone loss and disrupts vital organ function. Osteocytes comprise > 95% of the cellular component in bone tissue, produce a range of cytokines and signaling molecules, and influence bone and other organ function. In this review, we hypothesized that an elevated level of pro-inflammatory cytokines in inflammatory conditions affects osteocyte survival and function thereby possibly amplifying inflammation, and causing bone loss and non-bone clinical complications.

Recent Findings Several studies have reported that the elevated level of pro-inflammatory cytokines in inflammatory conditions alters osteocyte mechanosensitivity, causes osteocyte apoptosis, and modulates osteocyte-derived production of various inflammatory cytokines and signaling molecules. Cytokines and signaling molecules released from osteocytes affect surrounding bone cells and distant organ function in a paracrine and endocrine fashion.

Summary Inflammatory diseases including diabetes, chronic kidney disease, rheumatoid arthritis, and periodontitis affect osteocyte survival and function, and upregulate osteocyte-derived expression of sclerostin, RANKL, TNFα, FGF23, DKK1, and other signaling molecules.

Keywords Pro-inflammatory cytokines · Osteocytes · Inflammatory diseases · Endocrine function · Bone homeostasis, non-bone clinical complications

Introduction

Bone comprises around 15% of the total body weight, and osteocytes are the most prevalent cells in bone. The human body contains around 42 billion osteocytes with an average half life of 25 years [1]. The osteocyte is a highly mechanosensitive cell that translates mechanical stimuli to cellular signaling, regulates the activity of bone forming osteoblasts and bone resorbing osteoclasts, and maintains bone homeostasis [2, 3••]. Moreover, osteocytes regulate phosphate and calcium homeostasis, as well as work as endocrine cells targeting the kidney [4], lymphoid organ [5], heart [6••], and cancer [7••]. For this diverse function, osteocyte produces numerous cytokines and signaling molecules including sclerostin, receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), tumor necrosis factor-α (TNFα), interleukin (IL)-1β, IL-6, FGF23, DKK1, MEPE, PHEX, prostaglandins, nitric oxide, ATPs, and IGF-1 [8–11]. These cytokines and signaling molecules affect osteocytes, other bone cells and immune cells in bone niche, and distant organ function in autocrine, paracrine, and endocrine fashion. Sclerostin and DKK1 inhibit Wnt signaling, osteoblast activity, and bone formation [3••, 10••]. Osteocyte-derived RANKL enhances osteoclastogenesis, osteoclast activity, and bone resorption [3••]. Both local and systemic inflammation have been reported to affect osteocyte survival and activity. Inflammation occurs during a variety of pathological conditions such as rheumatoid arthritis [10••], periodontitis [12], inflammatory bowel disease [6••], kidney disease [13], chronic skin inflammation [14], bacterial infection [15••], cancer [16], obesity [17], diabetes [18, 19], injury [20], and other systemic inflammatory diseases. Inflammation-upregulated pro-inflammatory cytokines in the bone niche can easily enter the blood circulation, reach to distant organs, and cause systemic effects. Although a physiological level of pro-inflammatory cytokines is crucial for osteocyte function, an elevated level of pro-inflammatory cytokines modulates osteocyte survival, osteocyte-derived secretion of cytokines,

This article is part of the Topical Collection on *Osteocytes*

✉ Janak L. Pathak
janakpathak@163.com

[1] Key Laboratory of Oral Medicine, Guangzhou Institute of Oral Disease, Affiliated Stomatology Hospital of Guangzhou Medical University, Huangsha Avenue 39, Guangzhou 510140, China

and signaling molecules resulting in bone- and non-bone-related clinical complications. The osteocyte itself is a source of multiple pro-inflammatory cytokines such as TNFα, IL-6, IL-8, and IL-1β [10••]. An individual pro-inflammatory cytokine has the potential to upregulate osteocyte-derived cytokine production. Furthermore, a combination of multiple pro-inflammatory cytokines could synergistically elevate osteocyte-derived cytokine release [10••]. Under the influence of inflammation, osteocytes release pro-inflammatory cytokines, which modulate the function of bone cells in an autocrine and paracrine fashion. The osteocyte not only acts as a bone cell or an endocrine cell but also could amplify inflammation. Therefore, the osteocyte could be a potential therapeutic target to treat inflammation-related bone loss and other clinical complications.

In this review, we consider the osteocyte as a key player of inflammation-induced bone loss and non-bone clinical complications. We summarize the recent understanding of the effect of pro-inflammatory cytokines on osteocyte survival and function and osteocyte-mediated amplification of local and systemic inflammation during various inflammatory conditions. We further discuss the possible molecular mechanism of the osteocyte-mediated effects of pro-inflammatory cytokines on bone and non-bone clinical complications.

Inflammatory Diseases and Osteocytes

Obesity

Obesity, a multifactorial physical abnormality, is potentially preceding several clinical complications such as diabetes, hypertension, heart disease, metabolic syndrome, osteoarthritis, and osteoporosis [21]. Obesity causes chronic inflammation via upregulation of pro-inflammatory cytokines, such as TNFα, IL-1β, IL-6, RANKL, PTH, MCSF, and MCP1, which further activate the inflammatory cascade and affect various organ functions [17, 21–23]. Moreover, in obesity there is decreased bone formation and increased bone resorption via TNFα-induced upregulation of osteocyte-derived sclerostin and RANKL [23]. Pro-inflammatory cytokines inhibit osteoblast differentiation but enhance osteoclast formation and activity [21]. Recently, we reported that exogenous pro-inflammatory cytokine treatment upregulates TNFα, IL-1β, and IL-6 expression in human osteocytes [10••]. TNFα treatment in MLO-Y4 osteocytes upregulates sclerostin expression, and inhibition of NF-κβ signaling reversed this effect [23]. However, the effect of various obesity-induced inflammatory cytokines on osteocyte function has not been investigated yet [21]. Physical exercise causes mechanical stimulation of bone and muscle cells thereby improving muscle and bone quality. Whole body vibration on an obese child shows no effect on obesity but significantly reduces the serum sclerostin level [24•]. Multiple dietary components and exercise regimes have a synergistic anabolic effect on osteocyte function in both obese and non-obese conditions [25•]. The efficacy of a variety of dietary components or/and exercises on maintaining the physiological level of pro-inflammatory cytokines in obese patients is still an important subject to investigate. Future studies are necessary to thoroughly investigate the role of obesity-induced inflammation on osteocyte function and osteocyte-mediated signaling towards bone and other distant organs.

Periodontitis

Periodontitis is an inflammatory disease of periodontal tissue associated with other systemic inflammatory diseases, including rheumatoid arthritis, osteoporosis, and diabetes [12]. Periodontitis alone or in combination with other systemic disease causes alveolar and systemic bone loss. Similar to other inflammatory diseases, the effect of elevated levels of pro-inflammatory cytokines on osteocyte, osteoblast, and osteoclast function plays an important role in periodontitis-induced bone loss [26]. Osteocytes play a key role in development of periodontitis and periodontitis-induced alveolar bone loss [27••]. Periodontitis upregulates pro-inflammatory cytokine expression in alveolar osteocytes. Bone loss in diabetic rat with periodontitis correlates with elevated osteocyte-derived sclerostin, RANKL, TNFα, and IL-1β expression levels [27••, 28••, 29]. Yang et al. reported that sclerostin knockout rescues periodontitis-induced severe alveolar bone loss in mice [30]. Periodontitis-induced sclerostin elevates RANKL/OPG ratio and ERK1/2-MAPK in alveolar bone [30]. Periodontitis induces NF-κβ activation specifically in osteoblasts and osteocytes but not in gingival cells. Activated NF-κβ signaling enhances osteoclast activity and bone loss [31]. Inhibiting NF-κβ activation in osteoblast lineage cells reduces periodontal infection-induced RANKL expression, osteoclast numbers, and bone loss [31]. Mice with periodontitis and diabetes show higher alveolar bone loss, osteocyte-derived RANKL expression, osteoclast number, and osteoclast activity [27••]. The TNFα antagonist infliximab reduces sclerostin and RANKL expression in osteocytes, as well as prevents the alveolar bone loss in diabetic rats with periodontitis [28••]. In periodontitis, pro-inflammatory cytokine-induced upregulation of osteocyte-derived sclerostin, RANKL, and NF-κβ signaling causes periodontitis-induced bone loss. Most of the above-mentioned findings from the literature regarding periodontitis, inflammation, and osteocytes are obtained in an in vivo murine model. Clinical patient data are crucial to validate these findings. Therefore, future pre-clinical and clinical studies targeting alveolar osteocytes in periodontitis are essential to prevent alveolar bone loss and osteocyte-mediated amplification of inflammation.

Bacterial Infection

Both acute and chronic bacterial infections alter osteocyte function. Periprosthetic joint infection-induced osteomyelitis is difficult to treat and causes implant failure. *Staphylococcus aureus*, the most common pathogen in the periprosthetic joint, hides safely inside osteocytes and directly takes part in cellular and molecular activities [32••, 33]. The typical structure of osteocyte canaliculi provides a hiding place for *Staphylococcus aureus*, causing difficulties on osteomyelitis treatment [33]. Moreover, *Staphylococcus aureus* infection has been reported to upregulate osteocyte-derived expression of chemokines CCL5, CXCL9, CXCL10, and CXCL11 [33]. *Brucella abortus*, another common pathogen causing osteomyelitis, upregulates the expression of pro-inflammatory mediators including matrix metalloproteinase 2 (MMP-2), RANKL, TNFα, IL-6, and keratinocyte chemoattractant (KC) in osteocytes [15••]. Conditioned medium from *Brucella abortus*-infected osteocytes induces osteoclastogenesis via osteocyte-derived TNFα and RANKL upregulation. *Brucella abortus*-induced inflammation inhibits the expression of connexin 43 (Cx43) [15••], a protein needed for osteocyte survival, cell to cell communication, and normal structure and mechanical integrity of bone [15••, 34]. Moreover, conditioned medium from *Brucella abortus*-infected macrophages significantly reduces the osteocyte-derived expression of Cx43 and integrins, and induces osteocyte apoptosis [15••]. Deficiency of Cx43 increases the RANK/OPG ratio in osteocytes, bone resorption, osteoclast numbers, and osteocytes apoptosis [35–37]. Heat-killed *Brucella abortus* or exogenous IL-1β upregulates osseous FGF23 expression in mice [13]. Bacterial lipopolysaccharide (LPS), a major cause of inflammation during bacterial infection, is known to significantly increase the production of multiple pro-inflammatory cytokines such as IL-1β, TNFα, and IL-6 [38]. The pro-inflammatory cytokine IL-6 enhances osteocyte-mediated osteoclastogenesis via upregulation of RANKL and JAK2 activity in osteocytes [39]. Bacterial LPS-induced production of multiple inflammatory cytokines could disrupt bone homeostasis in an autocrine and paracrine fashion and further amplify the systemic inflammation. These literature reports show that the osteocyte produces pro-inflammatory cytokines, chemokines, and signaling molecules in response to bacterial infection, thereby disturbing bone cell function and bone homeostasis around the infected area. Moreover, the osteocyte acts as a hiding cave for bacteria causing difficulties to eliminate bacterial infection by traditional antimicrobial therapy. Development of novel therapeutic approaches targeting the osteocyte lacunae by, e.g., osteocyte-specific nanoparticles carrying a broad-spectrum of antimicrobial agents, is highly recommended to eliminate bacterial infection in osteomyelitis.

Diabetes and Aging

A high glucose level and advanced glycation end products (AGEs) are mediators of inflammation in diabetic patients and elderly people. High glucose and AGEs are associated with disrupted function of bone cells and osteoporosis. AGEs activate ERK1/2, P38, and STAT3 signaling via upregulation of osteocyte-derived IL-6 and VEGF-A secretion [18], and osteocytes apoptosis [18, 19]. A high glucose level and AGEs upregulate sclerostin expression in MLO-Y4 osteocytes [19]. A high glucose level increases the level of reactive oxygen species, TNFα expression, and osteocyte apoptosis [40]. Reactive oxygen species enhance sclerostin expression in osteocytes via TNFα upregulation and cause osteocyte apoptosis [40]. Signals from apoptotic osteocytes induce nearby osteocytes and macrophages to release growth factors and pro-inflammatory cytokines such as VEGF, RANKL, TNFα, IL-6, and IL-1β [41]. Diabetes aggravates periodontitis-induced alveolar bone loss [42]. It not only directly affects osteocyte survival and function but also escalates inflammation via the production of pro-inflammatory cytokines in osteocytes. This indicates a clear role of osteocytes in diabetes-induced bone loss and escalation of inflammation. Aging in mice severely reduces osteocyte-derived Cx43 [37, 43]. Old mice (21 months) have 95% less osteocyte-derived Cx43 compared to young mice (3.5 months) [37]. Decreased Cx43 expression reduces miR21 and promotes osteocyte apoptosis. Deletion of Cx43 robustly increases the osteocyte-derived expression ratio of RANKL/OPG and osteocyte-mediated osteoclastogenesis [37]. Oxidative stress is the main inducer of osteocyte apoptosis in diabetes and aging. Interestingly, Cx43 protects osteocytes from oxidative stress-induced cell death and its consequences in bone remodeling [43]. Development of therapeutic approaches to upregulate Cx43 expression in osteocytes could protect diabetes and/or aging-induced related bone loss.

Cancer

Various cancers including breast and lung are highly metastatic to the bone niche. Bone-metastasized cancer cells release signaling molecules and cytokines such as IL-6, IL-8, MCSF, MCP1, which could affect osteocyte function [44]. Primary cancer induces systemic inflammation, and bone-metastasized cancer amplifies local inflammation in the bone niche via direct interaction with osteoblasts and osteocytes, and causes osteolysis [7••]. Moreover, the bone niche acts as a fertile land to propagate metastasized cancer cells via interaction with various immune cells and bone cells including osteocytes. Conditioned medium (CM) collected from alendronate-

treated osteocytes inhibits breast cancer cell migration without affecting cancer cell viability [11]. Moreover, osteocyte-CM from both 2D and 3D cultures enhances migration and proliferation of MDA-MB231 breast cancer cells, and PC3 and DU145 prostate cancer cells [16]. Mechanical loading of osteocyte reduces IL-6 expression in osteocytes and ICAM expression on endothelial cells, which inhibits adhesion and trans-endothelial migration of breast cancer cells via crosstalk with osteoclasts and endothelial cells [45••]. Mechanically stimulated osteocytes inhibit breast cancer cell migration in vitro not only through direct signaling, but also via signaling from osteoclasts and endothelial cells [11, 45••]. This anti-metastatic potential of osteocyte-derived indirect signaling is particularly exciting since osteocytes are far away from metastasizing cancer cells. Therefore, the effect of different physical exercises on cancer bone metastasis could be an interesting research direction. The interaction of physical forces between tumor cells and the bone microenvironment has been reported to promote the growth of prostate cancer bone metastasis partly via upregulation of CCL5 and metalloproteinases in osteocytes [46]. Breast cancer has the highest rate of bone metastasis, but the role of the breast cancer-mediated physical forces inside the bone microenvironment in cancer bone metastasis is still unknown. Multiple myeloma, a plasma cell malignancy, has the highest incidence in bone among malignant diseases. Multiple myeloma cells induce osteocyte apoptosis causing upregulation of osteocyte-derived sclerostin and RANKL/OPG ratio [47]. Not only the cancer itself but also the anticancer chemotherapy has adverse effects on bone cell survival and activity. The combination of doxorubicin and cyclophosphamide therapy reduces osteocyte and bone lining cell numbers, and upregulates adipogenic factors and the expression of pro-inflammatory cytokines IL-1β and TNFα in the bone metaphysis and bone marrow [48••]. Therefore, adjuvant bone modifying therapy such as bisphosphonate along with anticancer drugs is commonly prescribed to minimize the catabolic effect of anticancer therapy on bone [49]. Various strategies targeting cancer cells, osteoblasts, and osteoclasts have been developed to treat bone-metastasized cancer [50–53]. However, only few reports are available on therapeutic approaches targeting osteocytes to prevent cancer bone metastasis and metastasis-induces osteolysis. Recently, Qiao and colleagues reported a unique design of zoledronic acid-loaded osteocyte targeting nanoparticles that attenuate early breast cancer metastasis to bone by inhibiting RANKL and SOST expression in osteocytes [7••]. Future studies on the role of osteocytes in cancer bone metastasis, cancer cell-osteocyte interaction, and cancer-induced osteocyte-mediated escalation of inflammation in the bone niche could guide the development of novel therapeutic agents targeting osteocytes to treat cancer bone metastasis and metastasis-induced osteolysis.

Chronic Kidney Disease

Sclerostin is mainly produced by osteocytes and is known to inhibit bone formation via inhibition of the Wnt signaling pathway [54•]. In chronic kidney disease (CKD), osteocyte-derived production of sclerostin and FGF23 is elevated [55]. Ito and colleagues reported that elevated serum phosphate alone or in combination with 1,25-dihydroxyvitamin D3 upregulates FGF23 mRNA expression in murine osteocytes [56]. DKK1 is another Wnt inhibitor reported to be elevated in CKD [57, 58]. A high level of sclerostin and DKK1 in CKD patients is associated with the pathogenesis of a bone disorder, vascular calcification, and cardiovascular events [55, 58–60]. Sclerostin could affect osteocyte-derived FGF23 and the expression of other osteocyte/osteoblast-related proteins PHEX, MEPE, and DMP1 [61, 62]. FGF23 is responsible for regulation of phosphate and vitamin D metabolism, as well as inhibits bone mineralization [63]. Increased circulating sclerostin serum level in kidney disease patients reflects decreased bone turnover [64]. Increased serum level of sclerostin in kidney disease positively correlates with serum 25-hydroxyvitamin-D, phosphorus, and TNFα [64]. The pro-inflammatory mediators TNFα, IL-1β, and bacterial LPS upregulate FGF23 expression in osteocytes [38]. TNFα and IL-1β-mediated upregulation of FGF23 in osteocytes in human bone is NF-kβ-dependent [38]. This indicates the possible role of inflammation-osteocyte crosstalk in systemic elevation of sclerostin, DKK1, and FGF23 in chronic kidney diseases.

Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease with local joint inflammation as well as systemic inflammation. Serum from patients with active rheumatoid arthritis (RA-serum) contains a cocktail of elevated levels of pro-inflammatory cytokines that possibly affects osteocyte function and causes bone loss. We recently reported that RA-serum enhances IL-1β, TNFα, SOST, and DKK1 gene expression in human bone chips cultured in ex vivo [10••]. Both sclerostin and DKK1 are negative regulators of bone regeneration [58]. In our ex vivo human osteocyte culture model [65], exogenous recombinant TNFα treatment enhances IL-1β, TNFα, IL-6, IL-8, and FGF23 gene expression. Exogenous recombinant IL-8 treatment enhances TNFα, IL-8, and FGF23 gene expression [10••]. A combination of exogenous recombinant IL-1β, IL-6, and TNFα treatment synergistically upregulates IL-1β, IL-6, and IL-8 gene expression, as well as enhances TNFα, OPG, SOST, and FGF23 gene expression [10••]. Moreover, RA-serum enhances osteocyte-mediated osteoclastogenesis and mechanical loading reverses this effect [66]. Sato and

colleagues reported correlation of elevated level of serum FGF23 in RA patient with disease activity and bone resorption [67]. However, the role of osteocytes on RA pathogenesis is still not fully understood. Development of RA in osteocyte-ablated mice could unravel the role of osteocytes in the pathogenesis of RA, RA-induced escalation of systemic inflammation, bone loss, and non-bone clinical complications.

Other Inflammatory Conditions

Inflammatory bowel disease causes systemic inflammation and enhances expression of TNFα, IL-6, sclerostin, and RANKL in osteocytes, as well as causes bone loss [6••]. The elevated serum levels of inflammatory cytokines in inflammatory bowel disease of childhood onset negatively interfere with the growth rate and bone metabolism regulation [68]. Psoriasis is a chronic skin inflammatory disease associated with increased serum IL17A level. IL17A, a pro-inflammatory cytokine, induces expression of IL-6, IL-1β, and IL-8 in cartilage, synoviocytes, macrophages, and bone cells, and has catabolic effects on bone [69]. A psoriasis mouse model showed IL17A-mediated inhibition of Wnt signaling in osteoblasts and osteocytes, as well as bone formation in vivo [14]. Psoriasis induces bone loss via inhibition of osteoblast/osteocyte function rather than activation of osteoclast function [14]. Spinal cord injury-related bone loss is caused by not only the loss of weight bearing and immobilization, but also by elevated circulating pro-inflammatory cytokines. Moreover, spinal cord injury induces osteocyte-derived expression of TNFα, IL-6, IL-17, IL-10, RANKL, and sclerostin, as well as causes bone loss [70]. This indicates a crucial role of loss of neural signaling in osteocyte-mediated amplification of inflammation in the bone niche and bone loss. Similarly, focal radiotherapy damages osteocytes and causes bone loss, via upregulation of sclerostin expression in osteocytes [71]. During knee injury, micro-cracks in bone initiate targeted remodeling via RANKL expression in osteocytes [20]. TCP wear particles can cause injuries of periprosthetic osteocytes and increase osteocyte-derived expression of sclerostin [72]. Similarly, titanium alloy wear particles enhance osteocyte autophagy and osteolysis, which could further amplify the inflammation and osteoclastogenesis [73]. Mechanical loading in a certain range has positive effects on osteocyte survival and function. However, implant-exerted excessive mechanical force on surrounding bone induces osteocyte apoptosis and the release of the osteocyte-derived cytokine RANKL [74]. Long-term application of anti-inflammatory drug glucocorticoids has adverse effects on bone. This adverse effect is mainly attributed to osteocyte apoptosis caused by glucocorticoids [75, 76]. Similarly, postmenopausal estrogen deficiency disrupts bone homeostasis and causes osteoporosis via upregulation of osteocyte-derived sclerostin and DKK1 [77, 78]. Pro-inflammatory

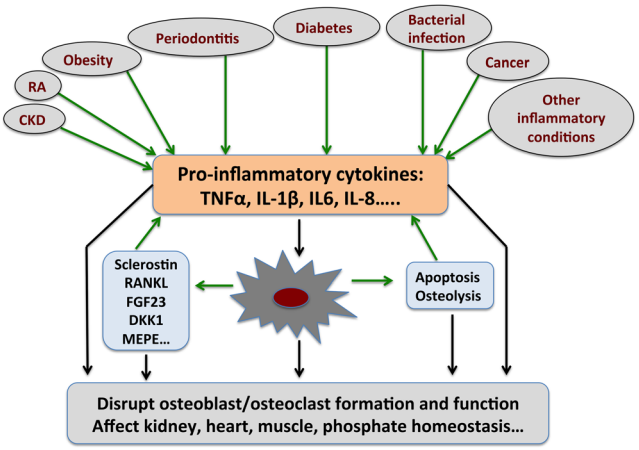

Fig. 1 Schematic diagram showing the effect of an elevated level of pro-inflammatory cytokines on osteocyte survival and function under various inflammatory conditions. Green arrow indicates stimilatory effect. RA, rheumatoid arthritis; CKD, chronic kidney diseases

cytokine-mediated effects on osteoblast and osteoclast formation and function in various inflammatory diseases are well documented compared to those affecting osteocyte survival and function. Since pro-inflammatory cytokines are able to modulate osteocyte survival, and paracrine and endocrine function (as demonstrated in Fig. 1), osteocytes could be a potential therapeutic target to alleviate the inflammation-induced bone-related and various non-bone clinical complications. We strongly recommend future research focusing on the interaction between pro-inflammatory cytokines and osteocytes and its role in bone homeostasis and other organ functions during various inflammatory diseases.

Conclusion

Recent studies on osteocytes indicate that a normal osteocyte function is crucial for healthy bone and normal function of the kidney, heart, lymphoid organs, and muscles. Elevated levels of pro-inflammatory cytokines in inflammatory conditions cause osteocyte apoptosis, the release of many inflammatory cytokines and signaling molecules that disturb bone homeostasis, and distant organ function, and could amplify local and systemic inflammation. Well-standardized physical exercise, antioxidant diet, and antibody against sclerostin or DKK1 have shown a beneficial effect on osteocyte survival and function in inflammatory conditions. However, only a few studies are available on the development of osteocyte-targeted therapy for cancer bone metastasis, chronic kidney disease, cardiovascular dysfunction, and muscle degeneration. Therefore, osteocytes should be considered as a potential future therapeutic target to treat pro-inflammatory cytokine-induced escalation of local and systemic inflammation, bone loss, and other non-bone-related diseases.

Funding Information This study was funded by National Natural Science Foundation of China (No. 81671029) and Guangzhou Science Technology and Innovation Commission (201704030024 and 201803040008).

Compliance with Ethical Standards

Conflict of Interest Miao Zhou, Shuyi Li, and Janak L. Pathak declare no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki Declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Buenzli PR, Sims NA. Quantifying the osteocyte network in the human skeleton. Bone. 2015;75:144–50.
2. Chen H, Senda T, Kubo KY. The osteocyte plays multiple roles in bone remodeling and mineral homeostasis. Med Mol Morphol. 2015;48(2):61–8.
3.•• Xiong J, Piemontese M, Onal M, Campbell J, Goellner JJ, Dusevich V, et al. Osteocytes, not osteoblasts or lining cells, are the main source of the RANKL required for osteoclast formation in remodeling bone. PLoS One. 2015;10(9):e0138189 **Used mice model with osteocyte specific deletion of RANKL to evaluate the role of osteocyte-derived RANKL in osteoclast formation during cancellous bone remodeling**.
4. Onal M, Carlson AH, Thostenson JD, Benkusky NA, Meyer MB, Lee SM, et al. A novel distal enhancer mediates inflammation-, PTH-, and early onset murine kidney disease-induced expression of the mouse Fgf23 gene. JBMR Plus. 2018;2(1):32–47.
5. Sato M, Asada N, Kawano Y, Wakahashi K, Minagawa K, Kawano H, et al. Osteocytes regulate primary lymphoid organs and fat metabolism. Cell Metab. 2013;18(5):749–58.
6.•• Metzger CE, Narayanan A, Zawieja DC, Bloomfield SA. Inflammatory bowel disease in a rodent model alters osteocyte protein levels controlling bone turnover. J Bone Miner Res. 2017;32(4):802–13 **Reported negative effect of inflammatory bowel disease on bone formation and bone mass via upregulation of osteocyte-derived TNFα, IL-6, RANKL, OPG, IGF-I and sclerostin**.
7.•• Qiao H, Cui Z, Yang S, Ji D, Wang Y, Yang Y, et al. Targeting osteocytes to attenuate early breast cancer bone metastasis by theranostic upconversion nanoparticles with responsive plumbagin release. ACS Nano. 2017;11(7):7259–73 **Designed osteocyte specific zoledronic acid-anchored nanoparticals that give a pH responsive release of the anticancer drug plumbagin. Zoledronic acid and plumbagin synergistically alleviate early bone metastasis of breast cancer and cancer bone metastasis-induced bone loss**.
8. Divieti Pajevic P, Krause DS. Osteocyte regulation of bone and blood. Bone. 2019;119:13–8.
9. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26(2):229–38.
10.•• Pathak JL, Bakker AD, Luyten FP, Verschueren P, Lems WF, Klein-Nulend J, et al. Systemic inflammation affects human osteocyte-specific protein and cytokine expression. Calcif Tissue Int. 2016;98(6):596–608 **Showed the effect of exogenous recombinant pro-inflammatory cytokines and RA-serum on upregulation of signaling molecules and cytokine expression in human osteocytes cultured in ex-vivo**.
11. Zhou JZ, Riquelme MA, Gao X, Ellies LG, Sun LZ, Jiang JX. Differential impact of adenosine nucleotides released by osteocytes on breast cancer growth and bone metastasis. Oncogene. 2015;34(14):1831–42.
12. Badran Z, Struillou X, Verner C, Clee T, Rakic M, Martinez MC, et al. Periodontitis as a risk factor for systemic disease: are microparticles the missing link? Med Hypotheses. 2015;84(6):555–6.
13. David V, Martin A, Isakova T, Spaulding C, Qi L, Ramirez V, et al. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. Kidney Int. 2016;89(1):135–46.
14. Uluckan O, Jimenez M, Karbach S, Jeschke A, Grana O, Keller J, et al. Chronic skin inflammation leads to bone loss by IL-17-mediated inhibition of Wnt signaling in osteoblasts. Sci Transl Med. 2016;8(330):330ra337.
15.•• Pesce Viglietti AI, Arriola Benitez PC, Gentilini MV, Velasquez LN, Fossati CA, Giambartolomei GH, et al. *Brucella abortus* invasion of osteocytes modulates connexin 43 and integrin expression and induces osteoclastogenesis via receptor activator of NF-kappaB ligand and tumor necrosis factor alpha secretion. Infect Immun. 2016;84(1):11–20 **Indicated osteocyte-derived connexin 43 and integrin as a regulator of osteocyte-mediated osteoclastogeneis during *Brucella abortus* infection in bone**.
16. Cui YX, Evans BA, Jiang WG. New roles of osteocytes in proliferation, migration and invasion of breast and prostate cancer cells. Anticancer Res. 2016;36(3):1193–201.
17. Denis GV, Sebastiani P, Bertrand KA, Strissel KJ, Tran AH, Slama J, et al. Inflammatory signatures distinguish metabolic health in African American women with obesity. PLoS One. 2018;13(5):e0196755.
18. Chen H, Liu W, Wu X, Gou M, Shen J, Wang H. Advanced glycation end products induced IL-6 and VEGF-A production and apoptosis in osteocyte-like MLO-Y4 cells by activating RAGE and ERK1/2, P38 and STAT3 signalling pathways. Int Immunopharmacol. 2017;52:143–9.
19. Tanaka K, Yamaguchi T, Kanazawa I, Sugimoto T. Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells. Biochem Biophys Res Commun. 2015;461(2):193–9.
20. Selvarajah L, Curtis AM, Kennedy OD. Bone microdamage in acute knee injury. Curr Rheumatol Rep. 2018;20(12):89.
21. Roy B, Curtis ME, Fears LS, Nahashon SN, Fentress HM. Molecular mechanisms of obesity-induced osteoporosis and muscle atrophy. Front Physiol. 2016;7:439.
22. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017;542(7640):177–85.
23. Baek K, Hwang HR, Park HJ, Kwon A, Qadir AS, Ko SH, et al. TNF-alpha upregulates sclerostin expression in obese mice fed a high-fat diet. J Cell Physiol. 2014;229(5):640–50.
24.• Tubic B, Zeijlon R, Wennergren G, Obermayer-Pietsch B, Marild S, Dahlgren J, Magnusson P, Swolin-Eide D. Randomised study of children with obesity showed that whole body vibration reduced sclerostin. Acta Paediatr 2018. **Reported the benefiical effect of whole body vibration-induced mechanical loading on mitigation of osteocyte-derived sclerostin production in obese children.**

25.• Willems HME, van den Heuvel EGHM, Schoemaker RJW, Klein-Nulend J, Bakker AD. Diet and exercise: a match made in bone. Curr Osteoporos Rep. 2017;15(6):555–63 **Emphasized the synergistic positive effect of multiple dietary components and physical exercise on osteocyte survival and bone health**.

26. Algate K, Haynes DR, Bartold PM, Crotti TN, Cantley MD. The effects of tumour necrosis factor-alpha on bone cells involved in periodontal alveolar bone loss; osteoclasts, osteoblasts and osteocytes. J Periodontal Res. 2016;51(5):549–66.

27.•• Graves DT, Alshabab A, Albiero ML, Mattos M, Correa JD, Chen SS, et al. Osteocytes play an important role in experimental periodontitis in healthy and diabetic mice through expression of RANKL. J Clin Periodontol. 2018;45(3):285–92 **Showed the importance of osteocyte-derived sclerostin on development of periodontitis and periodontitis-induced alveloar bone loss in healthy and diabetic experimental mice**.

28.•• Kim JH, Kim AR, Choi YH, Jang S, Woo GH, Cha JH, et al. Tumor necrosis factor-alpha antagonist diminishes osteocytic RANKL and sclerostin expression in diabetes rats with periodontitis. PLoS One. 2017;12(12):e0189702 **Reported TNFα as a main inducer of osteocyte-derived RANKL and sclerostin in diabetes accompanied with periodontitis**.

29. Kim JH, Lee DE, Woo GH, Cha JH, Bak EJ, Yoo YJ. Osteocytic sclerostin expression in alveolar bone in rats with diabetes mellitus and ligature-induced periodontitis. J Periodontol. 2015;86(8):1005–11.

30. Yang X, Han X, Shu R, Jiang F, Xu L, Xue C, et al. Effect of sclerostin removal in vivo on experimental periodontitis in mice. J Oral Sci. 2016;58(2):271–6.

31. Pacios S, Xiao W, Mattos M, Lim J, Tarapore RS, Alsadun S, et al. Osteoblast lineage cells play an essential role in periodontal bone loss through activation of nuclear factor-kappa B. Sci Rep. 2015;5:16694.

32.•• de Mesy Bentley KL, Trombetta R, Nishitani K, Bello-Irizarry SN, Ninomiya M, Zhang L, et al. Evidence of *Staphylococcus aureus* deformation, proliferation, and migration in canaliculi of live cortical bone in murine models of osteomyelitis. J Bone Miner Res. 2017;32(5):985–90 **Reported osteocyte canaliculi as a hiding cave for *Staphylococcus aureus* during osteomylitis. *Staphylococcus aureus* proliferates and migrates inside ostoecyte canaliculi without affecting osteocyte survival, and induces osteocyte-derived production of multiple cytokines and chmomokines**.

33. Yang D, Wijenayaka AR, Solomon LB, Pederson SM, Findlay DM, Kidd SP, Atkins GJ. Novel insights into *Staphylococcus aureus* deep bone infections: the involvement of osteocytes. MBio 2018, 9(2).

34. Hammond MA, Berman AG, Pacheco-Costa R, Davis HM, Plotkin LI, Wallace JM. Removing or truncating connexin 43 in murine osteocytes alters cortical geometry, nanoscale morphology, and tissue mechanics in the tibia. Bone. 2016;88:85–91.

35. Zhang Y, Paul EM, Sathyendra V, Davison A, Sharkey N, Bronson S, et al. Enhanced osteoclastic resorption and responsiveness to mechanical load in gap junction deficient bone. PLoS One. 2011;6(8):e23516.

36. Donahue HJ. Gap junctions and biophysical regulation of bone cell differentiation. Bone. 2000;26(5):417–22.

37. Davis HM, Pacheco-Costa R, Atkinson EG, Brun LR, Gortazar AR, Harris J, et al. Disruption of the Cx43/miR21 pathway leads to osteocyte apoptosis and increased osteoclastogenesis with aging. Aging Cell. 2017;16(3):551–63.

38. Ito N, Wijenayaka AR, Prideaux M, Kogawa M, Ormsby RT, Evdokiou A, et al. Regulation of FGF23 expression in IDG-SW3 osteocytes and human bone by pro-inflammatory stimuli. Mol Cell Endocrinol. 2015;399(C):208–18.

39. Wu Q, Zhou X, Huang D, Ji Y, Kang F. IL-6 enhances osteocyte-mediated osteoclastogenesis by promoting JAK2 and RANKL activity in vitro. Cell Physiol Biochem. 2017;41(4):1360–9.

40. Kang J, Boonanantanasarn K, Baek K, Woo KM, Ryoo HM, Baek JH, et al. Hyperglycemia increases the expression levels of sclerostin in a reactive oxygen species- and tumor necrosis factor-alpha-dependent manner. J Periodontal Implant Sci. 2015;45(3):101–10.

41. Jilka RL, Noble B, Weinstein RS. Osteocyte apoptosis. Bone. 2013;54(2):264–71.

42. Kim AR, Kim JH, Kim A, Sohn Y, Cha JH, Bak EJ, et al. Simvastatin attenuates tibial bone loss in rats with type 1 diabetes and periodontitis. J Transl Med. 2018;16(1):306.

43. Kar R, Riquelme MA, Werner S, Jiang JX. Connexin 43 channels protect osteocytes against oxidative stress-induced cell death. J Bone Miner Res. 2013;28(7):1611–21.

44. Chen YC, Sosnoski DM, Mastro AM. Breast cancer metastasis to the bone: mechanisms of bone loss. Breast Cancer Res. 2010;12(6):215.

45.•• Ma YV, Lam C, Dalmia S, Gao P, Young J, Middleton K, et al. Mechanical regulation of breast cancer migration and apoptosis via direct and indirect osteocyte signaling. J Cell Biochem. 2018;119(7):5665–75 **Showed direct osteocyte signaling as well as indirect osteocyte signaling through osteoclasts and endothelial cells as a regulator of breast cancer cell migration and apoptosis**.

46. Sottnik JL, Dai J, Zhang H, Campbell B, Keller ET. Tumor-induced pressure in the bone microenvironment causes osteocytes to promote the growth of prostate cancer bone metastases. Cancer Res. 2015;75(11):2151–8.

47. Delgado-Calle J, Bellido T, Roodman GD. Role of osteocytes in multiple myeloma bone disease. Curr Opin Support Palliat Care. 2014;8(4):407–13.

48.•• Fan C, Georgiou KR, Morris HA, McKinnon RA, Keefe DMK, Howe PR, et al. Combination breast cancer chemotherapy with doxorubicin and cyclophosphamide damages bone and bone marrow in a female rat model. Breast Cancer Res Treat. 2017;165(1):41–51 **Reported negative effect of anticancer drugs doxorubicin and cyclophosphamide on osteocytes**.

49. Heeke A, Nunes MR, Lynce F. Bone-modifying agents in early-stage and advanced breast Cancer. Curr Breast Cancer Rep. 2018;10(4):241–50.

50. Liu J, Zeng Y, Shi S, Xu L, Zhang H, Pathak JL, et al. Design of polyaspartic acid peptide-poly (ethylene glycol)-poly (epsilon-caprolactone) nanoparticles as a carrier of hydrophobic drugs targeting cancer metastasized to bone. Int J Nanomedicine. 2017;12:3561–75.

51. Li C, Zhang Y, Chen G, Hu F, Zhao K, Wang Q. Engineered multifunctional nanomedicine for simultaneous stereotactic chemotherapy and inhibited osteolysis in an orthotopic model of bone metastasis. Adv Mater 2017, 29(13).

52. Subia B, Dey T, Sharma S, Kundu SC. Target specific delivery of anticancer drug in silk fibroin based 3D distribution model of bone-breast cancer cells. ACS Appl Mater Interfaces. 2015;7(4):2269–79.

53. Swami A, Reagan MR, Basto P, Mishima Y, Kamaly N, Glavey S, et al. Engineered nanomedicine for myeloma and bone microenvironment targeting. Proc Natl Acad Sci U S A. 2014;111(28):10287–92.

54.• Yao W, Dai W, Jiang L, Lay EY, Zhong Z, Ritchie RO, et al. Sclerostin-antibody treatment of glucocorticoid-induced osteoporosis maintained bone mass and strength. Osteoporos Int. 2016;27(1):283–94 **Reported sclerostin-antibody as a therapeutic approach to ameliorate glucocorticoid-induced loss of bone mass and strength**.

55. Morena M, Jaussent I, Dupuy AM, Bargnoux AS, Kuster N, Chenine L, et al. Osteoprotegerin and sclerostin in chronic kidney disease prior to dialysis: potential partners in vascular calcifications. Nephrol Dial Transplant. 2015;30(8):1345–56.
56. Ito N, Findlay DM, Anderson PH, Bonewald LF, Atkins GJ. Extracellular phosphate modulates the effect of 1alpha,25-dihydroxy vitamin D3 (1,25D) on osteocyte like cells. J Steroid Biochem Mol Biol. 2013;136:183–6.
57. Moe SM, Chen NX, Newman CL, Organ JM, Kneissel M, Kramer I, et al. Anti-sclerostin antibody treatment in a rat model of progressive renal osteodystrophy. J Bone Miner Res. 2015;30(3):499–509.
58. Evenepoel P, D'Haese P, Brandenburg V. Sclerostin and DKK1: new players in renal bone and vascular disease. Kidney Int. 2015;88(2):235–40.
59. Kuipers AL, Miljkovic I, Carr JJ, Terry JG, Nestlerode CS, Ge Y, et al. Association of circulating sclerostin with vascular calcification in Afro-Caribbean men. Atherosclerosis. 2015;239(1):218–23.
60. Zeng C, Guo CY, Cai J, Tang CY, Dong Z. Serum sclerostin in vascular calcification and clinical outcome in chronic kidney disease. Diab Vasc Dis Res. 2018;15(2):99–105.
61. Sapir-Koren R, Livshits G. Osteocyte control of bone remodeling: is sclerostin a key molecular coordinator of the balanced bone resorption-formation cycles? Osteoporos Int. 2014;25(12):2685–700.
62. Asamiya Y, Yajima A, Shimizu S, Otsubo S, Tsuchiya K, Nitta K. Associations between the levels of sclerostin, phosphate, and fibroblast growth factor-23 and treatment with vitamin D in hemodialysis patients with low intact PTH level. Osteoporos Int. 2015;26(3):1017–28.
63. Guo YC, Yuan Q. Fibroblast growth factor 23 and bone mineralisation. Int J Oral Sci. 2015;7(1):8–13.
64. Pietrzyk B, Wyskida K, Ficek J, Kolonko A, Ficek R, Wiecek A, Olszanecka-Glinianowicz M, Chudek J. Relationship between plasma levels of sclerostin, calcium-phosphate disturbances, established markers of bone turnover, and inflammation in haemodialysis patients. Int Urol Nephrol 2018.
65. Shah KM, Stern MM, Stern AR, Pathak JL, Bravenboer N, Bakker AD. Osteocyte isolation and culture methods. Bonekey Rep. 2016;5:838.
66. Pathak JL, Bravenboer N, Luyten FP, Verschueren P, Lems WF, Klein-Nulend J, et al. Mechanical loading reduces inflammation-induced human osteocyte-to-osteoclast communication. Calcif Tissue Int. 2015;97(2):169–78.
67. Sato H, Kazama JJ, Murasawa A, Otani H, Abe A, Ito S, et al. Serum fibroblast growth factor 23 (FGF23) in patients with rheumatoid arthritis. Intern Med. 2016;55(2):121–6.
68. Ferreira P, Cavalcanti AS, Silva G. Linear growth and bone metabolism in pediatric patients with inflammatory bowel disease. J Pediatr (Rio J) 2018.
69. Amarasekara DS, Yu J, Rho J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. J Immunol Res. 2015;2015:832127.
70. Metzger CE, Gong S, Aceves M, Bloomfield SA, Hook MA. Osteocytes reflect a pro-inflammatory state following spinal cord injury in a rodent model. Bone. 2018;120:465–75.
71. Chandra A, Lin T, Young T, Tong W, Ma XY, Tseng WJ, et al. Suppression of sclerostin alleviates radiation-induced bone loss by protecting bone-forming cells and their progenitors through distinct mechanisms. J Bone Miner Res. 2017;32(2):360–72.
72. Yan JQ, Zhang Y, Liu FS, Cai TT, Tong KQ, Zhu C, et al. TCP wear particles causes injury of periprosthetic osteocytes in the mouse calvaria. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2018;34(1):83–7.
73. Wang Z, Deng Z, Gan J, Zhou G, Shi T, Wang Z, et al. TiAl6V4 particles promote osteoclast formation via autophagy-mediated downregulation of interferon-beta in osteocytes. Acta Biomater. 2017;48:489–98.
74. Takemura Y, Moriyama Y, Ayukawa Y, Kurata K, Rakhmatia YD, Koyano K. Mechanical loading induced osteocyte apoptosis and connexin 43 expression in three-dimensional cell culture and dental implant model. J Biomed Mater Res A 2018.
75. Guler-Yuksel M, Hoes JN, Bultink IEM, Lems WF. Glucocorticoids, inflammation and bone. Calcif Tissue Int. 2018;102(5):592–606.
76. O'Brien CA, Jia D, Plotkin LI, Bellido T, Powers CC, Stewart SA, et al. Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology. 2004;145(4):1835–41.
77. Liu M, Kurimoto P, Zhang J, Niu QT, Stolina M, Dechow PC, et al. Sclerostin and DKK1 inhibition preserves and augments alveolar bone volume and architecture in rats with alveolar bone loss. J Dent Res. 2018;97(9):1031–8.
78. Almeida M, Laurent MR, Dubois V, Claessens F, O'Brien CA, Bouillon R, et al. Estrogens and androgens in skeletal physiology and pathophysiology. Physiol Rev. 2017;97(1):135–87.