Opinion

Hypothesis on the pathogenesis of osteoarthritis

Abstract

Osteoarthritic joint changes represent a common phenomenon that, given its progression, is difficult to treat. Accordingly, knowledge about the pathogenesis of osteoarthritis is important. This article compares the mechanism of secondary bone healing with that of osteoarthritis, and can show clear parallels based on numerous study findings. This leads to the hypothesis that osteoarthritis corresponds to a mechanism of bone healing in the wrong place.

Keywords: osteoarthritis, pathogenesis, bone healing

Stephan Neumann
University of Goettingen, Germany

Correspondence: Stephan Neumann, Small animal Clinic, University of Goettingen, Burckhardtweg 2, D-37077 Goettingen, Germany, Tel 0049-551-3933387, Email sneuman@gwdg.de

Introduction

Degenerative joint changes are among the most common orthopedic diseases in humans and animals. As they progress, they can cause the complete destruction of the joint, including loss of function. Although there has been intensive research activity in this field, the findings on the pathogenesis of this disease are still incomplete.

The fact that joints ancylosis as a result of inactivity or final osteoarthritic changes, which creates a solid, bony connection between the articulated bones, and that, by contrast, insufficient bone healing after a fracture leads to pseudoarthrosis suggests that both mechanisms represent alternatives in bone and joint pathology.

It is these findings that gave rise to the hypothesis that the osteoarthritic joint changes reflect the organism's attempt to recreate a bony connection between the bone ends, pushed along by the mechanism of bone healing.

In simpler terms, osteoarthritis is bone healing in the wrong place!

The hypothesis is now to be demonstrated by comparative analyses of the mechanisms of bone healing and osteoarthritis. This involves looking at the phases of fracture healing with their biochemical and pathophysiological processes, and comparing them with osteoarthritis.

Statistical analysis

Data was collected and statistically analyzed using SPSS software version 20.0. Frequency, percentage, mean, standard deviation, correlation, one way ANOVA test and ROC curves was used to present the data; p-values <0.05 were considered statistically significant. Independent t test and chi square was used to assess the difference between the two study groups.

Bone healing

Bone healing consists of four phases

Inflammation, soft callus, hard callus and remodelling.[1] Depending on various factors, such as micro-movements which act during the healing phase, a distinction can be made between primary and secondary bone healing. According to the hypothesis, osteoarthritis would have to be a process equivalent to secondary bone healing.

In the following section, the phases of secondary bone healing will be compared with those of the pathogenesis of osteoarthritis

The first phase: The first phase of bone healing begins immediately after the trauma event. It is the "inflammatory phase", initiated by tissue destruction and the fracture hematoma. One can see destruction, bleeding and inflammatory cell infiltration in the affected tissue. In this phase of fracture healing, the concentration of proinflammatory cytokines, such as TNF-a, IL-1 and IL-6, increases in the fracture area.[2–4] Cytokines are secreted by macrophages and mesenchymal cells of the damaged tissue.[5,6]

This release of these cytokines plays a key role in the further pathophysiological processes. For example, TNF-a induces the migration of mesenchymal stem cells to the fracture area and causes their metamorphosis into osteoblast.[5,7–9] In the process, TNF-a acts via two receptor types, TNF-a Rec.1+2. Receptor 2 seems more significant for bone healing, which is underlined by the observation that progranulin, a growth factor of endochondral ossification, unfolds its effect in the development phase in relation to TNF-a Rec 2.[3,10]

IL-1 has a similar effect to TNF-a: it induces the endochondral bone formation by proliferating and differentiating pre-osteoblasts.[11,12] The growth-promoting effect of TNF-a and IL-1 is also reflected in the observation that TNF-a and IL-1 antagonists can suppress fracture healing.[13] The increased IL-6 concentration in a fracture area is seen as an indicator of its significance in connection with bone healing.[14] IL-6 increases osteoclastogenesis.[15] Tests on IL-6 knockout mice have shown that this is how IL-6 has a positive effect on callus mineralization.[16]

In summary, the function of proinflammatory cytokines in the early phase of fracture healing consists of attracting mesenchymal stem cells to the fracture area and, once there, inducing their differentiation into osteoblasts and subsequent mineralization.

During the osteoarthritic joint remodeling, there is also an increased secretion of proinflammatory cytokines. TNF-a is secreted by mononuclear inflammatory cells, synovial cells, chondrocytes and osteoblasts.[17] In the osteoarthritic joint, too, TNF-a acts via two receptors, with the effect mediated by TNF-a Rec.1 causing the destruction of the cartilage.[18] In the process, among other things, the formation of the extracellular matrix in the hyaline

cartilage tissue is suppressed.[19] Recently, the protective effect of progranulin and its effect via TNF-a Rec.2 have been demonstrated in the osteoarthritic joint.[20]

Like TNF-a, IL-1 is formed in the osteoarthritic joint primarily by mononuclear inflammatory cells, synovial cells, chondrocytes and osteoblasts.[21,22] IL-1 attaches to its target cells via two receptors[23,24] Significant effects of IL-1 include the suppression of the formation of the extracellular matrix by chondrocytes. Particularly inhibited is the synthesis of type II collagen and aggrecan.[25,26] In addition, IL-1 induces the synthesis of proteolytic enzymes, such as metalloproteinases (MMP1,2,13; ADAMTS), which have a further destructive effect on the cartilage components.[27,28] Finally, IL-1 can induce its own synthesis and that of other proinflammatory cytokines.[29,30] The importance of IL-1 in the etiology of osteoarthritis is further underlined by the observation that IL-1 antagonists, for example, in the form of IL-1 Rec, are expressed in the osteoarthritic joint at a reduced scale.[31]

Under the influence of IL-1 and TNF-a, IL-6 is formed by macrophages, chondrocytes, osteoblasts and adipocytes.[29,32,33] IL-6 acts via a receptor, and shows the same effects as IL-1 and TNF-a in the osteoarthritic joint with respect to reduced collagen production and synthesis of metalloproteinases.[34] In addition, IL-6 exhibits an effect on the subchondral bone, via activation of osteoclasts.[35] Also, in the mouse model, an effect was observed on the formation of osteophytes.[36] The IL-6 synovial and serum concentrations correlate in people with osteoarthritis with the radiographically detectable extent of joint changes.[37]

When comparing the cytokine secretions and effects in the fracture area and the osteoarthritic joint, it is possible to conclude that at the start of fracture healing, the inflammatory process, controlled by the proinflammatory cytokines, allows the building and formation of cells, such as osteoblasts and osteoclasts, to migrate to the fracture area. In the osteoarthritic joint, the initial focus is on the destruction of cartilage, which, following through on the hypothesis, can be seen as a preparatory measure to induce the migration of osteoblasts and osteoclasts from the surrounding tissue, especially the subchondral bone.

Apart from a comparable presence of cytokines in the fracture area and the osteoarthritic joint, one also encounters a similar situation in the mesenchymal stem cells, which underlines their significant role in both mechanisms. Mesenchymal stem cells demonstrate the ability to differentiate into different cell types, such as osteoblasts and chondroblasts.[38–40] In the fracture area, they represent the essential cells needed for healing. In the osteoarthritic joint, their proliferation behavior is reduced, except for osteogenic differentiation.[41] This suggests that the formation of bone material in the osteoarthritic joint, unlike cartilage, is not affected.

The second phase: The second phase of bone healing, the so-called soft callus, follows the inflammation phase. The mesenchymal stem cells that have migrated from the surrounding soft tissue, the cortical bone, bone marrow or via the blood differentiate into chondrocytes, which form the soft callus by means of forming the extracellular matrix.[42] The extracellular matrix is composed of collagens (I and II) and proteoglycans. TGF-ßs and BMPs are described as important factors in the formation of soft callus. Among the TGF-ß and BMP subfactors, TGF-ß1 and BMP-2, -4, -5, -6 seem to play a more significant role in the formation of soft callus.[6,43–46] Furthermore, angiogenic factors, such as VEGF, are involved in the callus formation by promoting the growth of new blood vessels in the fracture area.[47,48]

The same structural changes as with soft callus are also found during osteoarthritic joint remodeling, which includes connective tissue capsule thickening and osteophytic changes. A study on the gene expression of osteophytic cells found an expression pattern similar to the cells of soft callus.[49] The significance of cytokines and/ or growth factors TGF, BMP and VEGF in the osteoarthritic joint has also been described. An increased concentration of TGF-ß1 was detected in the synovia of osteoarthritic temporomandibular joints.[50] The effect of TGF-ßs in connection with capsular fibrosis has also been described.[51–53]

Increased concentration of BMPs was measured several times in osteoarthritic joints, and relations to the degree of destruction in the joint have been observed.[54–56] The significance of VEGF in the genesis of osteoarthritis is underscored by the observation that the inhibition of VEGF reduces the progression of osteoarthritis.[57] Thus, there are also structural and functional similarities between the second phase of fracture healing and osteoarthritis. While in fracture healing incipient stability is to be achieved through the soft callus, the osteoarthritic counterpart marks the beginning of joint stiffness and, thus, the preparatory phase of ancylosis.

The Third phase: The third phase of bone healing, the formation of hard callus, is also referred to as primary bone formation.[58] Supported by the high activity of osteoblasts, mineralizing bone matrix forms. This can involve both types of ossification, intramembranous and endochondral ossification. The BMPs play a key role in controlling these processes.[59,60] The significance of angiogenic factors at this time of bone formation has also been discussed.[61,62]

The active cells and growth factors in the formation of the hard callus can also be detected in the osteoarthritic joint, and in particular are tied to the formation of osteophytes.[63] It has also been debated whether BMPs have any significance in the capsule thickening.[64] This suggests that the osteophytic changes have a stabilizing effect on the joint to support the subsequent ossification. This theory is supported by the observation that osteophytes particularly occur at locations of the greatest movement.

Final phase: The final phase of bone healing is the formation of a physiological, spongiform bone tissue from the, as yet undifferentiated, hard callus, and is also called secondary bone formation.[58] Apart from osteoblasts, osteoclasts are particularly active in this phase. The formation of the latter is controlled by cytokines. The macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor κβ ligand (RANKL) are known as pro-osteoclastic factors. The former induces the differentiation of osteoblasts from hematopoietic stem cells, while the latter coordinates the bone resorption.[65,66] Osteoprotegerin, another factor, regulates the signal induction of RANKL.[67,68]

In addition to the forming cells, all factors described have been detected also in the osteoarthritic joint.[69,70] This, then, leads to the final joint remodeling followed by final ancylosis, and thus also to the reconstruction into stable bone tissue.

Conclusion

From the above, it follows that there is a clear congruence between osteoarthritic joint remodeling and bone healing. Apart from the factors described, there are certainly further factors that are active in both mechanisms, but it is unlikely, should they be discovered, that they will substantially alter the hypothesis presented herein. The hypothesis presented herein can serve as a basis for new treatment options for osteoarthritic joints.

Acknowledgments

None.

Conflicts of interest

The authors declare there is no conflict of interest.

References

1. Hunter J. The four stages of fracture healing. In: Palmer JF, editor. Collected Works. Vol 4. London, UK: Longman Rees; 1837.

2. Gerstenfeld LC, Cho TJ, Kon T, et al. Impaired fracture healing in the absence of TNF-alpha signaling: the role of TNF-alpha in endochondral cartilage resorption. *J Bone Miner Res*. 2003;18(9):1584–1592.

3. Ai-Aql ZS, Alagl AS, Graves DT, et al. Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. *J Dent Res*. 2008;87(2):107–118.

4. Marsell R, Einhorn TA. The biology of fracture healing. *Injury*. 2011;42(6):551–555.

5. Kon T, Cho TJ, Aizawa T, et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. *J Bone Miner Res*. 2001;16(6):1004–1014.

6. Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta super family during murine fracture healing. *J Bone Miner Res*. 2002;17(3):513–520.

7. Glass GE, Chan JK, Freidin A, et al. TNF-alpha promotes fracture repair by augmenting the recruitment and differentiation of muscle-derived stromal cells. *Proc Natl Acad Sci USA*. 2011;108(4):1585–1590.

8. Ulivi V, Tasso R, Cancedda R, et al. Mesenchymal stem cell paracrine activity is modulated by platelet lysate: induction of an inflammatory response and secretion of factors maintaining macrophages in a proinflammatory phenotype. *Stem Cells Dev*. 2014;23(16):1858–1869.

9. Gerstenfeld LC, Cullinane DM, Barnes GL, et al. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *J Cell Biochem*. 2003;88(5):873–884.

10. Zhao YP, Tian QY, Frenkel S, et al. The promotion of bone healing by progranulin, a downstream molecule of BMP-2, through interacting with TNF/TNFR signaling. *Biomaterials*. 2013;34(27):6412–6421.

11. Olmedo ML, Landry PS, Sadasivan KK, et al. Regulation of osteoblast levels during bone healing. *J Orthop Trauma*. 1999;13(5):356–362.

12. Mumme M, Scotti C, Papadimitropoulos A, et al. Interleukin-1β modulates endochondral ossification by human adult bone marrow stromal cells. *Eur Cell Mater*. 2012;24:224–236.

13. Perrien DS, Wahl EC, Hogue WR, et al. IL-1 and TNF antagonists prevent inhibition of fracture healing by ethanol in rats. *Toxicol Sci*. 2004;82(2):656–660.

14. Fazzalari NL. Bone fracture and bone fracture repair. *Osteoporos Int*. 2011;22(6):2003–2006.

15. Wallace A, Cooney TE, Englund R, et al. Effects of interleukin-6 ablation on fracture healing in mice. *J Orthop Res*. 2011;29(9):1437–1442.

16. Yang X, Ricciardi BF, Hernandez-Soria A, et al. Callus mineralization and maturation are delayed during fracture healing in interleukin-6 knockout mice. *Bone*. 2007;41(6):928–936.

17. Farahat MN, Yanni G, Poston R, et al. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis*. 1993;52(12):870–875.

18. Haas TL, Emmerich CH, Gerlach B, et al. Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and Is required for TNF-mediated gene induction. *Mol Cell*. 2009;36(5):831–844.

19. Roman-Blas JA, Jimenez SA. NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage*. 2006;14(9):839–848.

20. Zhao YP, Liu B, Tian QY, et al. Progranulin protects against osteoarthritis through interacting with TNF-α and β-Catenin signalling. *Ann Rheum Dis*. 2015;74(12):2244–2253.

21. Melchiorri C, Meliconi R, Frizziero L, et al. Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis Rheum*. 1998;41(12):2165–2174.

22. Massicotte F, Lajeunesse D, Benderdour M, et al. Can altered production of interleukin-1β, interleukin-6, transforming growth factor-β and prostaglandin E2 by isolated human subchondral osteoblasts identity two subgroups of osteoarthritic patients. *Osteoarthritis Cartilage*. 2002;10(6):491–500.

23. Caron JP, Fernandes JC, Martel-Pelletier J, et al. Chondroprotective effect of intraarticular injections of interleukin-1 receptor antagonist in experimental osteoarthritis: suppression of collagenase-1 expression. *Arthritis Rheum*. 1996;39(9):1535–1544.

24. Palmer G, Guerne PA, Mezin F, et al. Production of interleukin-1 receptor antagonist by human articular chondrocytes. *Arthritis Res*. 2002;4(3):226–231.

25. Stöve J, Huch K, Günther KP, et al. Interleukin-1β induces different gene expression of stromelysin, aggrecan and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes *in vitro*. *Pathobiology*. 2000;68(3):144–149.

26. Shakibaei M, Schulze-Tanzil G, John T, et al. Curcumin protects human chondrocytes from IL-1β-induced inhibition of collagen type II and β1-integrin expression and activation of caspase-3: an immuno-morphological study. *Ann Anat*. 2005;187(5-6):487–497.

27. Mengshol JA, Vincenti MP, Coon CI, et al. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappa B: differential regulation of collagenase 1 and collagenase 3. *Arthritis Rheum*. 2000;43(4):801–811.

28. Verma P, Dalal K. ADAMTS-4 and ADAMTS-5: key enzymes in osteoarthritis. *J Cell Biochem*. 2011;112(12):3507–3514.

29. Bender S, Haubeck HD, Van de Leur E, et al. Interleukin-1β induces synthesis and secretion of interleukin-6 in human chondrocytes. *FEBS Lett*. 1990;263(2):321–324.

30. Aigner T, McKenna L, Zien A, et al. Gene expression profiling of serum- and interleukin-1β-stimulated primary human adult articular chondrocytes - A molecular analysis based on chondrocytes isolated from one donor. *Cytokine*. 2005;31(3):227–240.

31. Fernandes JC, Martel-Pelletier J, Pelletier JP. The role of cytokines in osteoarthritis pathophysiology. *Biorheology*. 2002;39(1-2):237–246.

32. Ishimi Y, Miyaura C, Jin CH, et al. IL-6 is produced by osteoblasts and induces bone resorption. *J Immunol*. 1990;145(10):3297–3303.

33. Distel E, Cadoudal T, Durant S, et al. The infrapatellar fat pad in knee osteoarthritis: an important source of interleukin-6 and its soluble receptor. *Arthritis Rheum*. 2009;60(11):3374–3377.

34. Porée B, Kypriotou M, Chadjichristos C, et al. Interleukin-6 (IL-6) and/or soluble IL-6 receptor down-regulation of human type II collagen gene expression in articular chondrocytes requires a decrease of Sp1·Sp3 ratio and of the binding activity of both factors to the COL2A1 promoter. *J Biol Chem*. 2008;283(8):4850–4865.

35. Kwan Tat S, Padrines M, Théoleyre S, et al. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. *Cytokine and Growth Fac Rev*. 2004;15(1):49–60.

36. van de Loo FA, Kuiper S, van Enckevort FH, et al. Interleukin-6 reduces cartilage destruction during experimental arthritis: a study in interleukin-6-deficient mice. *Am J Pathol*. 1997;151(1):177–191.

37. Stannus O, Jones G, Cicuttini F, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cartilage*. 2010;18(11):1441–1447.

38. Jaiswal N, Haynesworth SE, Caplan AI, et al. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells *in vitro*. *J Cell Biochem*. 1997;64(2):295–312.

39. Mackay AM, Beck SC, Murphy JM, et al. Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng*. 1999;4(4):415–428.

40. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284(5411):143–147.

41. Murphy JM, Dixon K, Beck S, et al. Reduced chondrogenic and adipogenic activity of mesenchymal stem cells from patients with advanced osteoarthritis. *Arthritis Rheum*. 2002;46(3):704–713.

42. Dimitirou R, Tsiridie E, Giannoudis PV. Current concepts of molecular aspects of bone healing. Injury 2005;36(12):1392–1394.

43. Joyce ME, Roberts AB, Sporn MB, et al. Transforming growth factor-beta and the initiation of chondrogenesis and osteogenesis in the rat femur. *J Cell Biol*. 1990;110(6):2195–2207.

44. Yaoita H, Orimo H, Shirai Y, et al. Expression of bone morphogenetic proteins and rat distal-less homolog genes following rat femoral fracture. *J Bone Miner Metab*. 2000;18(2):63–70.

45. Tsuji K, Bandyopadhyay A, Harfe BD, et al. BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. *Nat Genet*. 2006;38(12):1424–1429.

46. Marsell R, Einhorn TA. The role of endogenous bone morphogenetic proteins in normal skeletal repair. *Injury*. 2009;40 Suppl 3:S4–S7.

47. Tsiridis E, Upadhyay N, Giannoudis P. Molecular aspects of fracture healing: which are the important molecules? *Injury*. 2007;38 Suppl 1:S11–S25.

48. Keramaris NC, Calori GM, Nikolaou VS, et al. Fracture vascularity and bone healing: a systematic review of the role of VEGF. *Injury*. 2008;39 Suppl 2:S45–S57.

49. Gelse K, Ekici AB, Cipa F, et al. Molecular differentiation between osteophytic and articular cartilage – clues for a transient and permanent chondrocyte phenotype. *Osteoarthritis Cartilage*. 2012;20(2):162–171.

50. Jiang Q, Qiu YT, Chen MJ, et al. Synovial TGF-β1 and MMP-3 levels and their correlation with the progression of temporomandibular joint osteoarthritis combined with disc displacement: A preliminary study. *Biomed Rep*. 2013;1(2):218–222.

51. Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol*. 1997;139(2):541–552.

52. Yang X, Chen L, Xu X, et al. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol*. 2001;153(1):35–46.

53. van der Kraan PM, Goumans MJ, et al. Age-dependent alteration of TGF-β signalling in osteoarthritis. *Cell Tissue Res*. 2012;347(1):257–265.

54. Goldring MB. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. *Best Pract Res Clin Rheumatol*. 2006;20(5):1003–1025.

55. Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. *Int Orthop*. 2007;31(6):773–781.

56. Albilia JB, Tenenbaum HC, Clokie CM, et al. Serum levels of BMP-2, 4, 7 and AHSG in patients with degenerative joint disease requiring total arthroplasty of the hip and temporomandibular joints. *J Orthop Res*. 2013;31(1):44–52.

57. Barranco C. Osteoarthritis: Animal data show VEGF blocker inhibits post-traumatic OA. *Nat Rev Rheumatol*. 2014;10(11):638.

58. Gerstenfeld LC, Cullinane DM, Barnes GL, et al. Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *J Cell Biochem*. 2003;88(5):873–884.

59. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors*. 2004;22(4):233–241.

60. Nakase T, Yoshikawa H. Potential roles of bone morphogenetic proteins (BMPs) in skeletal repair and regeneration. *J Bone Miner Metab*. 2006;24(6):425–433.

61. Brownlow HC, Reed A, Simpson AH. The vascularity of atrophic non-unions. *Injury*. 2002;33(2):145–150.

62. Peng H, Usas A, Olshanski A, et al. VEGF improves, whereas sFlt1 inhibits, BMP2-induced bone formation and bone healing through modulation of angiogenesis. *J Bone Miner Res*. 2005;20(11):2017–2027.

63. Blaney Davidson EN, Vitters EL, van Beuningen HM, et al. Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. *Arthritis Rheum*. 2007;56(12):4065–4073.

64. Scharstuhl A, Vitters EL, van der Kraan PM, et al. Reduction of osteophyte formation and synovial thickening by adenoviral over expression of transforming growth factor beta/bone morphogenetic protein inhibitors during experimental osteoarthritis. *Arthritis Rheum*. 2003;48(12):3442–3451.

65. Fan X, Biskobing DM, Fan D, et al. Macrophage colony stimulating factor down-regulates MCSF-receptor expression and entry of progenitors into the osteoclast lineage. *J Bone Miner Res*. 1997;12(9):1387–1395.

66. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature*. 1999;397(6717):315–323.

67. Blair JM, Zhou H, Seibel MJ, et al. Mechanisms of disease: roles of OPG, RANKL and RANK in the pathophysiology of skeletal metastasis. *Nat Clin Pract Oncol*. 2006;3(1):41–49.

68. Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res Ther*. 2007;9 Suppl 1:1.

69. Seitz M, Loetscher P, Fey MF, et al. Constitutive mRNA and protein production of Macrophage colony-stimulating factor. *Br J Rheumatol* 1994;33(7):613–619.

70. Tat SK, Pelletier JP, Velasco CR, et al. New perspective in osteoarthritis: the OPG and RANKL system as a potential therapeutic target? *Keio J Med*. 2009;58(1):29–40.