Original Research Paper

Volume - 13 | Issue - 02 | February - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Dentistry

THE THREE KEY PLAYERS IN OSTEOIMMUNOLOGY: RANK, RANKL AND OSTEOPROTEGERIN

Dr Priyanka T G	Second year post graduate Department of Periodontology Chettinad Dental College and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu,
Dr Ashwini Athul	Second year post graduate Department of Periodontology Chettinad Dental College and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu
Dr Srivainavi Arulmari	Third year post graduate Department of Periodontology Chettinad Dental College and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu
Dr Sophia Merlin Dawson	Third year post graduate Department of Periodontology Chettinad Dental College and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu
Dr Ashwath Balachandran*	M.D.S., Professor Department of Periodontology Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu,*Corresponding Author
Dr Shanmugam Muthukali	M.D.S., Professor Department of Periodontology Chettinad Dental College and Research Institute, Rajiv Gandhi Salai, Kelambakkam, Chengalpattu, Tamilnadu,

ABSTRACT The RANK, RANKL and OPG interaction plays a major role in bone resorption and remodelling. The history dates back to mid 1990s when the RANK/ RANKL interaction was found to mediate osteoblastic stromal cells to stimulate osteoclastic bone resorption. This interaction was found to induce several cytokines including the TNF superfamily, thereby activating the pathways of bone remodelling. The Osteoprotegerin (OPG) prevents the binding of RANKL to RANK, thereby preventing the excessive bone resorption. When there is an imbalance in the levels of RANK/RANKL/OPG, the metabolic activity of the bone cells gets altered and thus there is loss of balance between bone formation and resorption. Thus, studying the inter – relationship between RANK, RANKL and OPG becomes critical for assessing the osteoblastic and osteoclastic activity.

KEYWORDS : RANK, RANKL, Osteoprotegerin, TNF superfamily, bone remodelling

INTRODUCTION:

In higher vertebrates and mammals, bone remodelling and homeostasis play a crucial role in maintaining skeletal integrity throughout adulthood. Specialized bone cells, which appear to have opposed functions called bone production and bone resorption, regulate the maintenance of skeletal mass. As a result of disturbed or dysregulated osteoclastic activity, inflammatory bone disorders (such as rheumatoid arthritis, periodontitis, etc.) damage a significant amount of the mucosal surface and the osteoskeletal system in the human body. The differentiation, activation, and survival of osteoclasts and osteoclast precursors are all regulated by RANKL, RANK, and Osteoprotegerin, a recently discovered member of the TNF family of molecules.[1,2]

Osteoclasts are multinucleated cells that develop from hematopoietic stem cells that stimulate granulocyte and macrophage colony formation.[3] Numerous cytokines, such as IL-1, 6 and 11, macrophage colony stimulating factors, and calciotrophic hormones, such as PTH, 1,25 dihydroxy vitamin D3, and calcitonin, control the activity of osteoclastic cells. In conjunction with different cytokines and calciotrophic hormones, it has recently been demonstrated that members of the TNF and TNF receptor superfamily, receptor activated nuclear factor B ligand, receptor activated nuclear factor B, and Osteoprotegerin play a critical role in the formation and activation of osteoclasts.

Osteoclasts and stromal cells in the bone marrow express RANKL. Preosteoclasts and other cells of this lineage express the RANK receptor. By activating multiple transcription factors, the interplay of RANKL and RANK in the presence of macrophage colony stimulating factor, other cytokines, and calciotrophic hormones promotes osteoclastic development and differentiation.[4]

Osteoprotegerin is a decoy receptor that competes with RANKL for RANK and is generated by osteoblasts. This prevents bone resorption by preventing osteoclastic growth and differentiation.

PIONEER:

A crucial role for osteoblast/stromal cells in the control of osteoclast development and bone resorption was hypothesised by Rodan and Martin in 1981.[5] The osteoclast activating factor that completed the differentiation of precursors exposed to M-CSF was later the focus of intense investigation.[1]

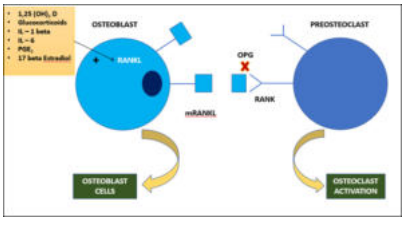

FIGURE 1: The RANKL ligand in the osteoblastic cells interact with RANK receptor present in preosteoclast resulting in conversion of preosteoclast to osteoclast.

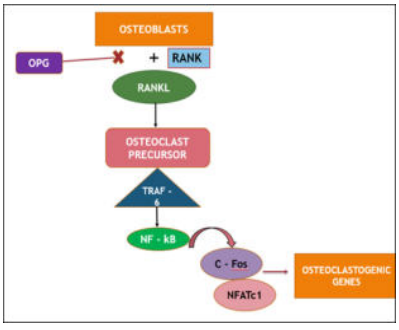

FIGURE 2: The OPG inhibits the RANK/ RANKL interaction thereby preventing activation of osteoclastogenic cells.

RANKL:

A type II homotrimeric trans membrane protein produced as a membrane bound and secreted protein and derived from membrane form via proteolytic cleavage or alternate splicing.[6] ADAM (a

disintegrin and metalloprotease domain) and matrix metalloproteases are required for RANKL proteolytic cleavage.[7] Most of the factors known to stimulate osteoclast formation and activity stimulate RANKL expression in osteoblast/stromal cells.

RANKL, like TNF, promotes the release of immature progenitors into the circulation. It is abundant in lymph nodes, the thymus, and the lungs. It is found in a variety of other tissues, including the spleen and bone marrow, at low levels. It is expressed by synovial cells and secreted by activated T cells in inflamed joints. Several studies have shown that RANKL is expressed by both T cells and B cells in human periodontal diseased gingival tissues.[8,9]

It was also discovered that the level of RANKL messenger RNA in the inflammatory cells of advanced periodontitis patients is higher than in those with moderate periodontitis or in the healthy group. Crotti et al used immunohistochemistry to show that periodontitis tissues had significantly higher levels of RANKL protein, which is associated with lymphocytes and macrophages. RANKL was abundantly expressed on CD3+T cells and CD20+B cells, but not on CD14+ monocytes. RANKL was expressed by more than 50% of T cells and 90% of B cells in diseased gingival tissues. In healthy gingival tissues, less than 20% of either B or T cells expressed RANKL.

Other studies found that CD4+ T cells were the most prevalent subset of cells invading gingival tissues of chronic periodontitis patients, and that they were the primary cells responsible for the greater tiers of RANKL found in these patients.[10] Induced bone resorption in periodontal tissues is both RANKL dependent and dependent on B7/CD28 costimulation between antigen presenting cells and T helper type I cells, as well as major histocompatibility complex-class II/T cell receptor engagement in antigen specific T cell activation. T cell RANKL expression is dependent on potassium channel Kvl.3 signalling, which is conditioned by the induction of local osteoclastogenesis.

RANK:

RANK is a TNF receptor superfamily homotrimeric transmembrane protein. At the protein level, it is expressed in fewer tissues than RANKL. Although no humans have been identified with RANK inactivating mutations or deletions, a deletion mutation occurred momentarily in a line of transgenic mice, resulting in mice with all of the characteristics of mice with RANK targeted deletion, affirming the emphasis of RANK for osteoclast formation. The steadily increasing osteolysis observed in certain patients with familial Paget's disease can be attributed to an activating mutation in exon I of RANK that causes an increase in RANKL - mediated nuclear factor B signalling and, as a result, an increase in osteoclast formation and activity. This has established the significance of this system in humans.[11]

OSTEOPROTEGERIN:

In addition to osteoblasts, Osteoprotegerin is secreted by cells in the heart, kidney, liver, and spleen. According to a recent study, B cells may be responsible for 64% of total bone marrow OPG production, and B cell deficient mice are consistently osteoporotic, indicating that B cells are a major source of OPG in normal mouse bone marrow.[12] Regardless of the conflicting data, when RANKL expression is upregulated, OPG expression is downregulated or not prompted to the same extent as RANKL, resulting in a shift in the RANKL/OPG ratio favouring osteoclastogenesis.[13]

The number and activity of osteoclasts can increase if the RANKL/OPG ratio changes owing to a rise in the former, a decrease in the latter, or a shift in both that results in a change in the ratio favouring RANKL (figure 2). OPG expression in osteoblasts is controlled not only by cytokines, hormones, and growth factors, but also by other mechanisms.

- The Wnt-cantenin pathway modulates osteoblastic bone formation and mesenchymal cell commitment to the osteoblastic lineage.
- Jagged1/Notch1 Signaling suppresses osteoclast formation both directly in osteoclast precursors and indirectly in stromal cells by influencing the OPG/RANKL expression ratio.

TRANSCRIPTION FACTOR ACTIVATION BY RANKL/RANK IN OSTEOCLASTS AND OCPs:

TNF receptor associated factor (TRAFs) binding to targeted positions in the cytoplasmic domain of RANK is an important preliminary step in downstream signalling after RANKL ligation. RANK is a transmembrane protein that, like other TNF family receptors, lacks innate protein kinase activating activity. TRAFs 2, 5, and 6 all attach to RANK, but only TRAF 6 appears to have vital functions in OCPs and osteoclasts, as deletion of only TRAF 6 leads to osteopetrosis.[14,15]

RANK/TRAF-mediated protein kinase signalling activates several signalling pathways. Four are directly involved in osteoclast formation.

- Inhibitor of Nfkβ kinase (IKK)/Nfkβ,
- C-jun-N-terminal kinase (JNK)/ activator protein 1(AP-1),
- c-myc,
- calcineurin/NFATc1

RANKL is produced by some of these cells when subjected to locally inflated proinflammatory cytokines and other inflammatory mediators. RANKL binds to RANK on the surface of osteoclast precursors and recruits the adaptor protein TRAP-6, resulting in Nfk activation via phosphorylation and inactivation of inhibitory kappa kinases (IKKs) and Nfk inhibitory proteins. This induces activation of C-Fos (figure 1).

Nfk and C-Fos interact with the NFATc1 promoter to initiate NFATc1 autoamplification and gene transcription, which mediates differentiation completion. TNF is produced in large quantities by accessory cells, macrophages/monocytes, and osteoclasts, in addition to RANKL expression. TNF binds to the TNF receptor, which activates C-Fos in osteoclast precursors via the Nfk and JNK pathways. TNF also stimulates the expression of TNF and IL-1 by OCPs. IL-1 does not stimulate C-Fos, but in OCPs where C-Fos has been stimulated, such as by RANKL or TNF, OL-1 can directly induce osteoclastogenesis. This results in an increase in osteoclast formation via the same NFATc1-activated mechanism as RANKL.

RANKL/RANK expression is required for OCP differentiation in both physiologic (via osteoblastic cells) and pathological (via accessory cells and OCP themselves) conditions, whereas TNF signalling appears to be important in inflammatory bone diseases such as periodontitis. Overall, NFATc1 is considered to be the primary regulator of osteoclast formation.[16]

POTENTIAL LYMPHOCYTE-MEDIATED PERIODONTAL BONE RESORPTION INTERVENTION STRATEGIES:

New periodontal disease treatments must focus on the significant immune cell contribution to bone resorption. Most current periodontal disease treatments focus solely on mechanical procedures, such as scaling, root planing, and surgery, and ignore immune cells. As a result, inhibiting RANKL production by activated immune cells will provide a number of effective therapeutic approaches for periodontal bone loss. Approaches that specifically target RANKL production by activated immune cells may include:

(i) Interference with co-stimulatory molecules that are required for RANKL production by activated immune cells,

(ii) Control of signaling pathways regulating RANKL expression in immune cells, and

(iii) Down-regulation of lymphocyte RANKL expression by manipulation of cytokine balance.

(iv) Physiological blockade of RANKL–RANK interaction,

(v) Deactivation of the tumor necrosis factor-a converting enzyme that releases soluble RANKL from membrane-bound RANKL expressed on activated lymphocytes, and

(vi) Local application of pharmaceutical compounds, such as bisphosphonates, designed to interfere with lymphocyte-derived RANKLmediated osteoclastogenesis.

(I) Interference with co-stimulatory atoms that are required for RANKL generation by enacted safe cells:

Blocking B7 CD28 interaction with cytotoxic T-lymphocyte antigen-4-immunoglobulin (CTLA4-Ig) inhibitory co-stimulation meddling with T-cell-mediated periodontal bone resorption. Total T-cell

actuation requires the transmission of two signals, the primary from the T-cell receptor and another from co-stimulatory atoms. B7-1 (CD80) and B7-2 (CD86) are costimulatory atoms that have a place to the immunoglobulin superfamily. B7-1 is found on enacted B cells, T cells, and macrophages. B7-2 is communicated constitutively on dendritic cells and memory B cells. Both B7-1 and B7-2 are able of official the immunoglobulin superfamily receptors CD28 and cytotoxic T lymphocyte antigen 4. Cytotoxic T-lymphocyte antigen-4 isn't communicated on the cell surface by default; or maybe, it is quickly up-regulated after CD28 ligation and T-cell activation.

Cytotoxic T-lymphocyte antigen-4 is not expressed on the cell surface by default; rather, it is rapidly up-regulated after CD28 ligation and T-cell activation. As a result, CTLA4 is involved in the counter-regulatory mechanism for suppressing excessive T-cell immune responses. T-cell receptor and CD28 stimulation can increase the expression of RANKL in T cells. In situations where RANKL expressed by activated T cells is the primary cause of periodontal bone loss, local administration of cytotoxic T-lymphocyte antigen-4 immunoglobulin may be beneficial in interfering with immune cell mediated inflammatory bone resorption.

Anti-CD40 ligand blockade of CD40 ligand interaction interferes with B-cell-mediated periodontal bone resorption:

CD40 is a tumour necrosis factor receptor family member that is expressed in B cells, macrophages, dendritic cells, endothelial cells, and fibroblasts. CD40 ligand (CD154) is almost exclusively expressed on activated T cells but not on naive T cells or any other cell type. CD40 ligand ligation induces bidirectional signalling in both APC and T cells, initiates humoral and cellular immune responses, including the expression of numerous cytokines and chemokines, and results in Ig class switching from IgM to IgG.

The 29kDa outer membrane protein of A. actinomycetemcomitants and lypopolysaccharides induced periodontal bone loss, which was accompanied by increased production of soluble RANKL and inflammatory cytokines in gingival tissues, according to studies. Furthermore, data suggest that activated B cells may be involved in RANKL augmentation in T helper type 1 cell elicited periodontal bone resorption via CD40 ligand/CD40 interaction. Blocking costimulatory molecules, such as CD40 or CD40 ligand, which are expressed on nearly all T cells and B cells, respectively, may disrupt general immune responses to exogenous pathogens. Thus, it remains to be seen whether local gingival injections of anti-CD40 ligand could be a viable intervention strategy for periodontal bone resorption.

(ii) Control of signaling pathways controlling expression of RANKL in immune cells:

Ca2+/Calcineurin pathway:

- Because RANKL is produced by T cells after TCR stimulation and its transcriptional over expression is partially Ca2+ dependent, under expression of Ca2+ signalling in activated T cells is expected to reduce RANKL expression.
- Despite the risks, local administration of cyclosporin A with TGF- or citrate has the potential to improve or suppress periodontal bone resorption.

Potassium (K+) channel pathway:

- Of the two potassium channels, Kv1.3 and IKCa1, that are involved in a variety of functions in cells found in inflammatory lesions, potassium channel receptor Kv1.3 is important in the expression of RANKL by T cells.
- Kv1.3 inhibition with kaliotoxin (scorpion venom) suppressed RANKL expression in activated T cells, showing remarkable results in decreasing experimental alveolar bone resorption.
- In addition to T cells, Kv1.3 and IKCa1 are involved in B-cell activation. Before using Kv1.3 blockers for immunotherapy, it is critical to assess their side effects.

Tumor necrosis factor-receptor associated factor 6 (TRAF6) pathway:

RNA interference, a newly discovered, powerful molecular mechanism that can be used to engineer gene-specific silencing in mammalian tissues, was used to reduce tumour necrosis factor-receptor mRNA and protein expression. Well before technique can be recognised as a promising clinical therapy for these diseases, including periodontitis, potential technical hurdles must be overcome.

Toll-like receptor pathway:

Co-activation of the Toll-like receptor 4 (lipopolysaccharide) and 9 (cytosine-guanosine) signalling pathways can regulate RANKL production by immune cells and may provide useful molecular targets for therapeutic purposes.

(iii) Down-regulation of lymphocyte rankl expression by manipulation of cytokine balance:

It is proposed that modulating the local cytokine profile allows for the inhibition of bone loss, which may be beneficial in the treatment of periodontal bone resorption. This can be accomplished through local expression of immunosuppressive cytokines like IL-10, IL-4, and IL-13, or through local injections of inhibitors that reduce the secretion of bone resorptive cytokines like TNF-, IL-1, IL-6, and IL-17.

(iv) RANKL osteoclastogenesis inhibition: Osteoprotegerin-Fc and anti-RANKL antibody:

a) Osteoprotegerin-Fc:

Osteoprotegerin-Fc is a chimeric protein that combines OPG with the Fc portion of human IgG. While retaining the RANKL-neutralizing activity of Osteoprotegerin, the Fc portion conjoined to the protein provides additional benefits by facilitating protein purification in the manufacturing process and increasing protein stability in vivo. OPG-Fc has also been shown to be an effective inhibitor of B-cell-mediated periodontal bone resorption. However, genuine anti-OPG antibodies may result from OPG-Fc administration, posing a potential and unwelcome risk to patients.

Anti-RANKL antibody:

More recent studies have extended this general immunological blockade of the RANKL-RANK interaction by using an investigational monoclonal antibody (Denosumab) that hinders the NFKB ligand and thus neutralises RANKL. When compared to OPG-Fc, this antibody has the following advantages: it is more potent, with stronger declines in bone turnover markers at lower doses and a longer duration of action at equivalent doses. It was seen to increase bone mineral density and decrease bone resorption in postmenopausal women.

(vii) Inactivation of the enzyme (TACE) responsible for the release of soluble RANKL:

An inhibitor of the tumour necrosis factor-a-converting enzyme (TACE or ADAM-17), which plays a key role in RANKL ectodomain shedding, can partially reverse it. Other MMPs, such as MMPs-1, -3, -7, -14, and ADAM-19, can also cleave RANKL, but at a lower rate. Local administration of TACE inhibitor galardin downregulated T-cell mediated periodontal bone resorption in a rat model. TANCE can cleave off TNF superfamily proteins in addition to releasing soluble RANKL. Immunological inhibition of TACE activity, either by antibody or inhibitor, could be a potential treatment for immune cell-mediated periodontal bone resorption.

(viii) Pharmaceutical compound application - potency and side effects of bisphosphonates:

The biological mechanism underlying bisphosphonate-mediated bone loss reduction is derived from its inhibition of osteoclast proliferation and differentiation, as well as induction of apoptosis in mature osteoclasts. Bisphosphonates not only react to osteoclasts, but also to osteoblasts, decreasing their expression of RANKL, with an associated increase in TACE in osteoblasts. Instead of IV administration, bisphosphonates can be administered via nasal, subcutaneous, and intramuscular injections, implants, and targeted osteotrophic delivery systems, enhancing bioavailability.

CONCLUSION:

The discovery of the RANK/RANKL/OPG system has greatly expanded our comprehension of the processes underlying bone remodelling. This system is crucial in the pathophysiological mechanisms that underpin inflammatory bone disorders. The discovery of this system has resulted in the development of novel therapeutic options for the treatment of skeletal disorders.

REFERENCES:

1) Simonet, W.S., D.L. Lacey, C.R. Dunstan, M. Kelley, M.-S. Chang, R. Luethy, H.Q. Nguyen, S. Wooden, L. Bennett, T. Boone, et al. 1997. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 89:309–319
2) Lacey, D.L., E. Timms, H.-L. Tan, M.J. Kelley, C.R. Dunstan, T. Burgess, R. Elliott, A. Colombero, G. Elliott, S. Scully, et al. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 93:165–176
3) Suda T, Takahashi N,Martin TJ. Modulation of Osteoclast Differentiation. Endocr Rev. 1992;13:66–80.

4) Blair JM, Zheng Y & Dunstan CR 2007 RANK ligand. International Journal of Biochemistry & Cell Biology 39 1077–1081. (doi:10.1016/j.biocel.2006. 11.008
5) Rodan GA, Martin TJ: Role of osteoblasts in hormonal control of bone resorption: a hypothesis. Calcif Tissue Int 1981, 33: 349-351.
6) Ikeda T, Kasai M, Utsuyama M, Hirokawa K. Determination of three isoforms of the receptor activator of nuclear factor-kappaB ligand and their differential expression in bone and thymus. Endocrinology. 2001 Apr;142(4):1419-26. doi:10.1210/endo.142.4.8070. PMID: 11250921.
7) Hikita A, Yana I, Wakeyama H, Nakamura M, Kadono Y, Oshima Y, Nakamura K, Seiki M, Tanaka S. Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-kappaB ligand. J Biol Chem. 2006 Dec 1;281(48):36846-55. doi: 10.1074/jbc.M606656200. Epub 2006 Oct 3. PMID: 17018528.
8) Choi Y, Woo KM, Ko SH, Lee YJ, Park SJ, Kim HM, Kwon BS. Osteoclastogenesis is enhanced by activated B cells but suppressed by activated CD8(+) T cells. Eur J Immunol 2001: 31: 2179–2188.
9) Liu D, Xu JK, Figliomeni L, Huang L, Pavlos NJ, Rogers M, Tan A, Price P, Zheng MH. Expression of RANKL and OPG mRNA in periodontal disease: possible involvement in bone destruction. Int J Mol Med 2003: 11: 17–21
10) Vernal, Rolando et al. "High Expression Levels of Receptor Activator of Nuclear Factor-Kappa B Ligand Associated With Human Chronic Periodontitis Are Mainly Secreted by CD4+T Lymphocytes." Journal of Periodontology 77.10 (2006): 1772–1780. Web.
11) Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM. Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet. 2000 Jan;24(1):45-8. doi: 10.1038/71667. PMID: 10615125.
12) Li Y, Toraldo G, Li A, Yang X, Zhang H, Qian WP, Weitzmann MN. B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. Blood. 2007 May 1;109(9):3839-48. doi: 10.1182/blood-2006-07-037994. Epub 2007 Jan 3. PMID: 17202317; PMCID: PMC1874582.
13) Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. Cytokine Growth Factor Rev. 2004 Dec;15(6):457-75. doi: 10.1016/j.cytogfr.2004.06.004. PMID: 15561602.
14) Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev. 1999 Apr 15;13(8):1015-24. doi: 10.1101/gad.13.8.1015. PMID: 10215628; PMCID: PMC316636.
15) Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J. Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells. 1999 Jun;4(6):353-62. doi: 10.1046/j.1365-2443.1999.00265.x. PMID: 10421844.
16) Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell. 2002 Dec;3(6):889-901. doi: 10.1016/s1534-5807(02)00369-6. PMID: 12479813.