ORIGINAL RESEARCH PAPER

Periodontology

THE RANK AND RANKL NEXUS – MOLECULAR ASPECTS

KEY WORDS: RANK, RANKL, Osteoclasts, NFKbeta, TNF superfamily

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ABSTRACT

RANKL are the chemical mediators secreted by the resident cells of the connective tissue and in the mileau of periodontium they are released during the process of bone turnover either for bone remodelling during orthodontic tooth movement, normal masticatory force bearing, age related changes or for bone resorption due to pathological reasons where plaque influences the cells of inflammation to release PGE2, IL-1 and TNF alpha. These RANKL act on the circulating pre osteoclasts or monocyte macrophage cells which under the influence of M-CSF will start to colacase to form multinucleated osteoclasts that will resorb the bone. Thus, for these various changes to occur in the macrophages and preosteoclasts there will be a need of transcription factors like NFK□, NFATc1, cFos and others. To understand intracellular mechanisms when RANKL binds onto the trimeric receptor RANK which leads to a better understanding of therapeutic management to deal with pathological bone destructive disease, so we have consolidated this narrative review.

INTRODUCTION:

RANKL the ligand has received much attention in the field of inflammatory diseases either osteoarthritis or periodontitis, if we trace the origin of these cytokines, they lead us to various resident cells of mesenchymal origin that secrete RANKL which binds onto the cells of the haemopoetic linage leading to a cascade of events that cause a bearing on bone morphology. This interaction of RANKL with RANK or OPG has been studied under the umbrella of the tern osteoimmunology. With the advent of better diagnostic abilities, the basis of many inflammatory conditions of the bone are better understood now, even the pathogenesis of osteoporosis has been elucidated much better in the recent years, one major bone of contention was the treatment of rheumatoid arthritis and we now know that the involvement of immunological mediators in the joint and synovial fluid has been a major cause leading to interaction of bone cells causing more bone resorption and inflammation. Researchers then took their knowledge of osteoporosis and arthritis to explain the pathogenesis of periodontal disease which shares the same environment.[1]

The earlier understanding of bone was that of a static tissue that only helps in protecting the organs and provide a rigid structural framework. But, with further knowledge of physiological functions of bone we know that this mineralized tissue is a dynamic one that helps in to maintain the level of electrolytes, this reservoir is maintained by the actions of osteoclasts (OCL) that resorb the bone which comes from the linage of hemopoietic cells and osteoblasts which lays down the bone that on the other hand comes from the mesenchymal cells. This interaction between the bone resorption and bone deposition which is better known as bone coupling as explained by ten cate and others can take place during physiological process for maintenance of electrolyte balance and bone remodelling at the load bearing areas as is explained during the orthodontic tooth movement process. It is also well known by now that PTH and fibroblast growth factor are also responsible for the signalling process involved in the bone turnover. However, the pathological process of bone resorption has been a bane to tackle with, which involves the signalling of pre osteoclasts with the cytokines that enhance and initiate their differentiation and proliferation and among them the main culprit lies in the overexpression of RANKL which binds onto the NFK beta receptor present on the precursor cells of osteoclasts causing their activation.

Osteoblasts are mononucleated cells that synthesize the organic matrix of bone. Osteoblasts arise from pluripotent stem cells, which are of mesenchymal origin in the axial and appendicular skeleton and of ectomesenchymal origin (neural crest cells that migrate in mesenchyme) in the head. Although osteoblasts are differentiated cells, both preosteoblasts and osteoblasts can undergo mitosis during prenatal development and occasionally during postnatal growth.[2]

Bone as we know is a vascular connective tissue that provides a rigid framework to support all the vital organs, even though it is rigid it shows remarkable flexibility when subjected to external forces and internal changes in the metabolic activity that can bring about real dynamic nature to the fore. But, to think of this nature of bone it's important to understand the mechanisms that is involved in the process of this turnover and for getting into the knowledge of theses process we need to know the basic molecular nature of the bone. Microscopically as we look into the cross actions of bone, they show lamellar structure which can be either concentric or interstitial lamella arranged circumferentially, each lamellae is again traversed by a central volkman canal, osteocytes make up the major part of the cells that are present in them in a lacunae connected by canaliculi. Osteoblasts are present around the periosteum that line the outermost layer of the bone and endosteum line the inner most layer of the bone housing the bone marrow or fatty adipose tissue in certain bones. Now, let's look at the cells that make up the bone.[2]

1. Osteoblasts:

Osteoblasts are plump, cuboidal cells (when very active) or slightly flattened cells that are primarily responsible for the production of the organic matrix of bone. They exhibit abundant and well-developed protein synthetic organelles. At the light microscopic level, the Golgi complex characteristically appears as a clear, paranuclear area that can be defined easily after cytochemical reactions for Golgi enzymes. The secretory matrix products of osteoblasts include type I collagen which is the most abundant and dominant part of organic matrix, small amounts of other collagens including type V and type XII; proteoglycans; and several noncollagenous proteins. The spherical and cylindrical distentions of the Golgi complex are believed to represent different stages of procollagen processing and packaging. The typical elongated, electron-dense, collagen-containing secretory granules release their contents primarily along the surface of the cell apposed to forming bone.[29]

The organic deposition formedof osteoblasts in the form of matrix, which is not yet calcified, is termed as osteoid. The lag phase between osteoid deposition and its mineralization is approximately 10 days. Differentiation of MSCs into osteoblast depends on the expression of two key transcription factors, Runx2 and its target Osterix, in response to external stimuli. Parathyroid hormone (PTH), Prostaglandin E2 (PGE2), insulin-like growth factor (IGF)-1, bone morphogenic proteins (BMPs), and Wingless and Int-1 (Wnt) proteins are key stimuli for osteoblast differentiation PGE2 is an important anabolic factor for bone and induces the expression of bone sialoprotein and alkaline phosphatase in mesenchymal cells. BMPs and transforming growth factor (TGF)-β, which shares structural similarities with BMPs, foster osteoblast differentiation by activating intracellular SMADs. Wnt proteins belong to a family of highly conserved signaling molecules which are potent stimulators of osteoblast differentiation. Wnt proteins bind to receptors on mesenchymal cells such as Frizzled and low-density lipoprotein receptor-related protein 5 (LRP5), eliciting activation and nuclear translocation of β-catenin, thereby inducing transcription of genes involved in osteoblast differentiation.[2]

2. Osteocytes: Osteocytes are by far the most abundant cell type found within the bone. One cubic millimeter of bone contains up to 25,000 osteocytes, which are considerably connected with each other and the bone surface by small tubes (canaliculi), constituting a large and dense communication network inside the bone. The surface of this network of lacunae containing the osteocytes along with the canaliculi containing the interconnecting filaments of the osteocytes covers an area of 1000 to 4000 square meters. Osteocytes are derived from osteoblasts, which are subsequently entrapped in the bone matrix. One of the most interesting products of the osteocyte is sclerostin, a secreted molecule that binds LRPs and blocks Wnt-stimulated bone formation (4). Consistent with its function as an inhibitor of bone formation, sclerostin overexpression leads to low bone mass, whereas its deletion leads to increased bone density and strength.[3]

3. Osteoclasts: Bone resorption is as much important as bone deposition, as the bone is a vascular connective tissue its turnover is maintained by resorption and deposition in the process called as 'Bone coupling'. Resorption is the process which precedes deposition and is accomplished by multinucleated giant cells called osteoclasts. In several human diseases (e.g. malignant hypercalcemia and postmenopausal osteoporosis) enhanced bone resorption is the key pathophysiological event, and therapies for these diseases are currently based on its inhibition.[4] In contrast, some rare genetic disorders are manifested as decreased resorption and lead to osteopetrosis. A promyeloid precursor can differentiate into either an osteoclast, a macrophage or a dendritic cell, depending on whether it is exposed to receptor activator of NF-kB ligand (RANKL; also called tumor necrosis factor-related activation-induced cytokine (TRANCE), osteoprotegerin ligand (OPGL) or osteoclast differentiation factor (ODF)), macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GMCSF), respectively. Simonet et al. in 1997 [5] found that several cells and tissues produce a soluble factor, osteoprotegerin (OPG), that strongly inhibits osteoclast formation in vitro and in vivo. More recently, several groups demonstrated that bone marrow stromal cells and osteoblasts produce membrane bound and soluble RANKL/TRANCE/OPGL/ODF, an important positive regulator of osteoclast formation. The inhibitory effect of OPG on the osteoclast differentiation is due to the fact that it can prevent the binding of RANKL to its receptor, RANK.[6]

a. Resorption requires cellular activities: migration of the osteoclast to the resorption site, its attachment to bone, polarization and formation of new membrane domains, dissolution of hydroxyapatite, degradation of organic matrix, removal of degradation products from the resorption lacuna, and finally either apoptosis of the osteoclasts or their return to the non-resorbing stage.[7]

b. After migration of the osteoclast to a resorption site, a specific membrane domain, the sealing zone, forms under the osteoclast. The plasma membrane attaches tightly to the bone matrix and seals the resorption site from its surroundings. The molecular interactions between the plasma membrane and the bone matrix at the sealing zone are still unknown. Several lines of evidence have shown, however, that integrins play an important role in early phases of the resorption cycle. At least four different integrins are expressed in osteoclasts: avb3, avb5, a2b1 and avb. The role of avb3 has received much attention, because antibodies against avb3, as well as RGD-containing peptides such as echistatin and kistrin, are effective inhibitors of bone resorption both in vitro and in vivo. avb3 is highly expressed in osteoclasts and is found both at the plasma membrane and in various intracellular vacuoles. However, the precise function(s) of avb3 in resorbing osteoclasts remains unknown; the integrin could play a role both in adhesion and migration of osteoclasts and in endocytosis of resorption products. The latter possibility is supported by the observation that high amounts of avb3 are present at the ruffled border and by recent data from receptor binding assays showing that denatured type I collagen has a high affinity for avb3.[8]

c. Resorbing osteoclasts (in contrast to non-resorbing osteoclasts) are highly polarized cells. Current data suggest that resorbing osteoclasts contain not only the sealing zone but also at least three other specialized membrane domains: a ruffled border, a functional secretory domain and a basolateral membrane. As the osteoclast prepares to resorb bone, it attaches to the bone matrix through the sealing zone and forms another specific membrane domain, the ruffled border. The ruffled border is a resorbing organelle, and it is formed by fusion of intracellular acidic vesicles with the region of plasma membrane facing the bone. During this fusion process much internal membrane is transferred, and forms long, finger-like projections that penetrate the bone matrix. The characteristics of the ruffled border do not match those of any other plasma membrane domain described. Although facing the extracellular matrix, it has several features that are typical of late endosomal membranes. Several late endosomal markers, such as Rab7, Vtype H-ATPase and lgp110, but not lgp120, are densely concentrated at the ruffled border.[9]

d. The main physiological function of osteoclasts is to degrade mineralized bone matrix. This involves dissolution of crystalline hydroxyapatite and proteolytic cleavage of the

organic matrix, which is rich in collagen. Before proteolytic enzymes can reach and degrade collageneous bone matrix, tightly packed hydroxyapatite crystals must be dissolved. It is now generally accepted that the dissolution of mineral occurs by targeted secretion of HCl through the ruffled border into the resorption lacuna. This is an extracellular space between the ruffled border membrane and the bone matrix, and is sealed from the extracellular fluid by the sealing zone. After solubilization of the mineral phase, several proteolytic enzymes degrade the organic bone matrix, although the detailed sequence of events at the resorption lacuna is still obscure. Two major classes of proteolytic enzymes, lysosomal cysteine proteinases and matrix metalloproteinases (MMPs), have been studied most extensively. The high levels both of expression of MMP-9 (gelatinase B) and cathepsin K and of their secretion into the resorption lacuna suggest that these enzymes play a central role in the resorption process.[10]

e. After matrix degradation, the degradation products are removed from the resorption lacuna through a transcytotic vesicular pathway from the ruffled border to the functional secretory domain, where they are liberated into the extracellular space. Recent studies have suggested that tartrate-resistant acid phosphatase (TRAP), a widely used osteoclast marker, is localised in the transcytotic vesicles of resorbing osteoclasts, and that it can generate highly destructive reactive oxygen species able to destroy collagen. This activity, together with the co- localisation of TRAP and collagen fragments in transcytotic vesicles, suggests that TRAP functions in further destruction of matrix-degradation products in the transcytotic vesicles.[11]

f. The primary factors that stimulate bone resorption through osteoclasts include parathyroid hormone, vitamin D3, interleukin-1, interleukin-6, tumor necrosis factor a, and transforming growth factor-a, whereas calcitonin, transforming growth factor- b, estrogen and interferon-g inhibit osteoclastic bone resorption. While osteoclasts have receptors for calcitonin and estrogen, as well as for most cytokines, vitamin D3 and parathyroid hormone affect osteoclasts indirectly through receptors on pre- osteoblasts, osteoblasts and lining cells.[12]

g. Notably, the effects of vitamin D3 can also be mediated by the bone matrix protein osteopontin, which is strongly upregulated by the seco- steroid. Another bone matrix protein, bone sialoprotein, can also regulate osteoclast activity through the avb3 integrin which was originally characterized as a vitronectin receptor.

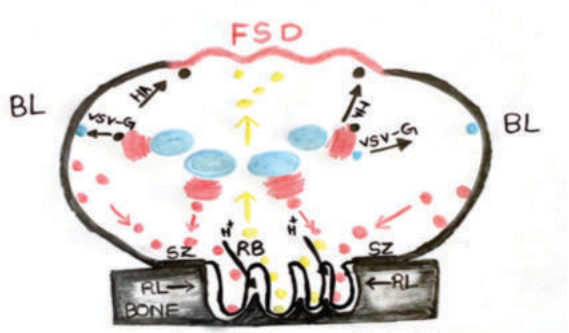

Fig 1: Schematic view of a bone resorbing osteoclast.

Extensive vesicular trafficking involving several specific membrane domains is a hallmark of actively resorbing cells. BL, basolateral domain (blue); FSD, functional secretory domain (red); SZ, sealing zone (green); RB, ruffled border (black). Brown vesicles illustrate vesicular pathways from the trans-Golgi network and the basolateral membrane to RB, and yellow vesicles illustrate the transcytotic route from the RB to the FSD. Vesicular pathways from the trans-Golgi network to the apical (black vesicles) and basolateral (blue vesicles) membrane domains are shown (for references see text). HA, haemagglutinin; VSV-G, vesicular stomatitis virus G protein. RL, resorption lacuna (white).[13]

After a brief description of bone cells given above its now important to note that for the bone remodelling to occur osteoclasts are the cells that need to be recruited at the site and for this to occur the cytokines responsible are RANKL and MCSF encoded by Tnfsf11), which are expressed by osteoblast-lineage cells [14] or osteocytes,[15] are required for the differentiation of osteoclast precursors into mature osteoclasts.[16] The released RANKL binds to its receptor RANK (encoded by Tnfrsf11a), which is expressed via M-CSF stimulation on the surface of osteoclast precursor cells. also negatively regulated by osteoprotegerin (encoded by Tnfrsf11b), which is a soluble decoy receptor for RANKL that obstructs RANKL binding to RANK.[17] RANK is a member of the tumor necrosis factor receptor (TNFR) superfamily that lacks intrinsic enzymatic activity that is required for activating downstream signalling molecules. Thus, RANK transduces intracellular signals by recruiting adaptor molecules such as TNFR associated factors (TRAFs), which then activate mitogen activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB).[18] RANK signalling is controlled via the spatiotemporal regulation of downstream signalling, which results in the commitment of monocyte/macrophage precursor cells to the osteoclast lineage and the activation of mature osteoclast

Rank Signaling And Traf Adaptor Proteins

RANKL binding to RANK induces the trimerization of RANK and the recruitment of an adaptor protein, TRAF6, via three TRAF6-binding sites in its C-terminal cytoplasmic tail that TRAF6 is the major adaptor protein responsible for mediating signaling cascades that are activated by RANKL.[19] Activated TRAF6 stimulates NF-κB activity by activating the IκB kinase (IKK) complex, either via atypical protein kinase C (aPKC) or TGFβ-activated kinase 1 (TAK1)-dependent phosphorylation, which is a process that requires IKKγ (also called NEMO) ubiquitination to achieve optimal activation.[20] The scaffolding protein p62 binds to TRAF6 and interacts with aPKCs, resulting in the formation of a multimeric protein complex that regulates IKKb. Transcription factors such as NF-.B and AP-1 are essential downstream targets of the early RANKL signaling pathway. The recruitment of adaptor proteins also leads to the activation of MAPKs such as c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase, and Akt/PKB.

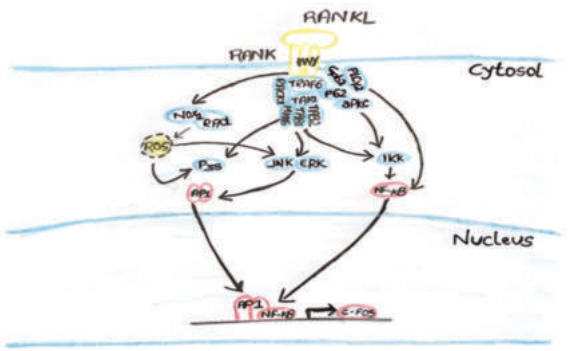

Fig. 2. Initiation of RANK signaling is mediated by TRAF6. RANK stimulation by RANKL binding induces the recruitment and activation of a major adaptor protein, TRAF6. TRAF6 activates NF-κB either by interacting with p62 and aPKC or via TAK1 phosphorylation to regulate the IKK complex. Gab2 and PLCγ2 are other molecules that are required for NF-κB activation; they are recruited to RANK and activated. In addition, TRAF6 complexes with TAK1-TABs or TAK1-RACK1-MKK6 to facilitate the activation of MAPKs such as p38, JNK, and ERK. ROS produced by the RANK-TRAF6-Rac1-Nox1 cascades regulate MAPK activation. Activation of NF-κB and

MAPKs leads to the induction of c-Fos at the initial stage of RANK signaling. RANK, receptor activator of nuclear factor-κB; RANKL, receptor activator of nuclear factor-κB ligand; TRAF6, TNF receptor-associated factors 6; NF- κB, nuclear factor-κB; aPCK, atypical protein kinase C; IKK, IκB kinase; TAK1, TGFβ-activated kinase 1; Gab2, growth factor receptor-bound protein 2 (Grb2)-associated binder-2; PLCγ2, phospholipase Cγ2; TAB, TAK1-binding protein; RACK1, receptor for activated C kinase 1; MAPKs, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; NOX1, NADPH oxidase 1; AP-1, activator protein-1; NFATc1, nuclear factor of activated T-cells cytoplasmic 1. (Adapted from Park JH et al).[21]

Costimulatory Signaling

After the initial induction of NFATc1 by NF-.B, c-Fos/AP-1, and NFATc2 RANK signaling functions together with the activation of immunoglobulin-like receptor/immunoreceptor tyrosine-based activation motif (ITAM) signaling (Fig. 2), which leading to the robust amplification of NFATc1 and its translocation to the nucleus where it binds with DNA in combination with other transcription factors to upregulate the transcription of NFATc1 target genes.[20] NFATc1 may be a master transcription factor in osteoclastogenesis and numerous in vivo studies have demonstrated the essential role of NFATc1 in osteoclast formation. In addition to RANK signaling, immunoglobulin-like receptors such as triggering receptor expressed in myeloid cells-2 (TREM-2) and osteoclast-associated receptor (OSCAR) transduce NFATc1 induction signals. These immune receptors have short cytoplasmic tails and are associated with adaptor proteins such as DNAX-activation protein 12 (DAP12) or the Fc receptor common subunit (FcR.), which contains ITAM.[22]

When ITAM is tyrosine phosphorylated, possibly induced by RANKL stimulation, a complex is formed and contains the tyrosine kinases such as Bruton's tyrosine kinase (Btk)/Tec, adaptor molecules B cell linker protein (BLNK), and Src homology 2 domain containing leukocyte protein of 76 kDa (SLP-76), and this complex may integrate both RANK and ITAM downstream signaling. Thus, This suggests that Btk and Tec are essential for osteoclastogenesis and that these kinases regulate NFATc1 activation via PLC.2 and the Ca2+ signaling pathway.

In response to RANKL stimulation, RANK forms a signaling complex that comprises EEIG1, Gab2, PLC.2, and Btk/Tec, suggesting that EEIG1 is an adaptor protein and a linker protein that integrates RANK and ITAM signaling. Along with RANK signaling, ITAM signaling leads to the long-term induction of PLC.2 activation. Activated PLC.2 produces inositol-1,4,5-triphosphate (IP3) by digesting phosphatidylinositol-4,5-bisphosphate, and IP3 then binds to IP3 receptors on the surface of the endoplasmic reticulum (ER). Thus, the Ca2+ stored in ER is released into the cytoplasm, and Ca2+ oscillation is induced.

In the absence of stimulation by RANKL, NFATc1 is heavily phosphorylated by active glycogen synthase kinase- 3ß (GSK-3ß) and is localized in the cytoplasm. After RANK signaling induces GSK-3ß phosphorylation at its inhibitory serine residue (Ser9 of GSK-3ß), GSK-3ß is inactivated. This enables NFATc1 dephosphorylation via calcineurin, followed by its translocation into the nucleus In the nucleus, NFATc1 induces the expression of target genes.

Akt overexpression in osteoclast precursors strongly induces NFATc1 expression and its nuclear localization owing to the inactivation of GSK-3ß via its increased phosphorylation, A recent study demonstrated that PKCß controls the activity of NFATc1 by inactivating GSK-3ß, thereby resulting in osteoclastogenesis (Shin et al., 2014).[23] These results suggest that the GSK-3ß/NFATc1 signaling cascade plays important roles in RANK signaling during osteoclast differentiation. This cooperation of RANK signalling with ITAM associates molecules is shown in the Fig no.2 below.

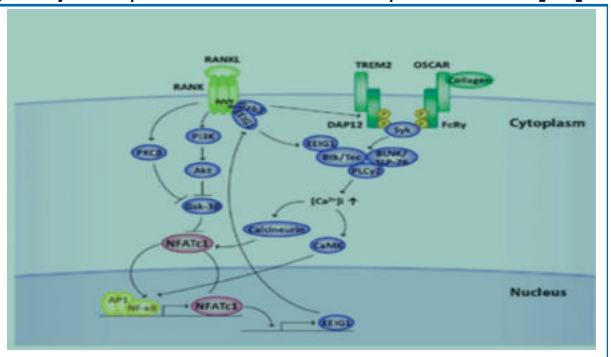

Fig. 3. Cooperation of RANK signaling with costimulatory receptors. RANK signaling cooperates with immunoglobulin-like receptor/ITAM signals such as TREM-2/DAP12 and OSCAR/FcRγ, thereby leading to the amplification and translocation of NFATc1. When ITAM is tyrosine phosphorylated, Btk/Tec and BLNK/SLP-76 form a complex with PLCγ2 to activate PLCγ2 and Ca2+ signaling. EEIG1 induced by NFATc1 associates with Gab2 via the IVVY motif in RANK and then activates Btk/Tec followed by PLCγ2, suggesting that EEIG1 integrates RANK and ITAM signaling. Ca2+ oscillation induces calmodulin and calcineurin activation to regulate the nuclear translocation and amplification of NFATc1. The subcellular localization of NFATc1 is influenced by the phosphorylation of serine residues regulated by Gsk-3β, which is inhibited by either PI3K-Akt signaling or PKCβ. ITAM, immunoreceptor tyrosine-based activation motif; TREM-2, triggering receptor expressed in myeloid cells-2; DAP12, DNAX-activation protein 12; OSCAR, osteoclast-associated receptor; FcRγ, Fc receptor common γ subunit; Btk, Bruton's tyrosine kinase; BLNK, B cell linker protein; SLP-76, Src homology 2 domain-containing leukocyte protein of 76 kD; EEIG1, early estrogen-induced gene 1; Gsk-3β, Glycogen synthase kinase-3β; PI3K, phosphoinositide 3-kinase; PKCβ, protein kinase Cβ. (Adapted from Park JH et al).[21]

Late Stage Of Signaling In Osteoclast Differentiation

In the late stage of RANK signaling, amplified NFATc1 activates and induces osteoclast-specific genes that encode proteins related to osteoclast differentiation, fusion, and function. Moreover, positive or negative regulators of NFATc1 are enhanced or suppressed during osteoclast differentiation, The expression of negative regulators is downregulated during osteoclast differentiation in response to RANK. All of the negative factors are downregulated by the transcription repressor B-lymphocyte-induced maturation protein 1 (Blimp1).[22] Blimp1 is induced by RANKL stimulation during osteoclast differentiation, and mice that lack Blimp1 in their osteoclasts exhibit osteopetrotic phenotypes owing to osteoclastogenesis aggravation. Several proteins such as dendritic cell-specific transmembrane protein (DC-STAMP), vacuolar proton pump subunit Atp6v0d2, and c-Src substrate Tks5 are involved in osteoclast cell-cell fusion; their expression is regulated by NFATc1 together with PU.1, microphthalmia-associated transcription factor, and c-Fos. aVß3, the integrin vitronectin receptor, binds to vitronectin in the bone matrix, leading to bone resorption via the recruitment of c-Src tyrosine kinase. which recruits DAP12 and SLP-76, which form an adaptor protein complex that activates the small Rho family GTPases, including Rac.[23]

These interactions lead to the formation of the ruffled border membrane via the fusion of lysosomal secretory vesicles with the cytoplasmic membrane (Zou et al., 2007). To resorb bone, H+ ions are pumped out through the ruffled border and form HCl with Cl-, which demineralizes the bone matrix, and various hydrolases such as cathepsin K, CLC-7 chloride channel, and TRAP, which are NFATc1 target genes, are activated. Kim et al. (2009) suggested that the cytoplasmic motif of RANK is specifically involved in osteoclast formation and function by regulating the actin cytoskeleton and survival of osteoclasts.

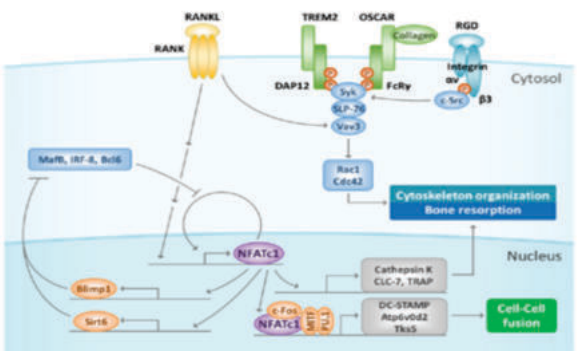

Fig. 4. Late stage of RANK signaling. Amplified NFATc1 induces its target genes to regulate osteoclast differentiation, cell fusion, and function. NFATc1 represses negative regulators such as MafB, IRF-8, and Bcl6 during osteoclast differentiation by inducing Blimp1 and Sirt6, which act as transcription repressors. In addition, NFATc1 cooperates with other transcription factors such as MITF, c-Fos, and PU.1 to regulate osteoclast fusion by inducing DC-STAMP, Atp6v0d2, and Tks5. The αVβ3 integrin signal activates c-Src by binding to vitronectin. Next, c-Src phosphorylates Syk, which recruits DAP12 and SLP-76 to form a complex with Vav3. This complex activates Rac1 or Cdc42 and regulates the cytoskeleton organization that is important for regulating bone resorption. MafB, v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B; IRF-8, interferon regulatory factor-8; Bcl6, B cell lymphoma 6; Blimp1, B-lymphocyte-induced maturation protein 1; Sirt6, sirtuin 6; MITF, microphthalmia-associated transcription factor; DC-STAMP, dendritic-cell-specific transmembrane protein; TRAP, tartrate-resistant acid phosphatase; Cdc42, cell division cycle 42. . (Adapted from Park JH et al).[21]

So, it is clear now that many studies both invitro and animal studies have shown that the action of osteoclast right from its differentiation from pre osteoclast or from the cells of monocyte – macrophage linage require the activation of RANK by RANKL and the costimulation of TREM2 and its related receptor family for subsequent activation of cascade molecules leading to transcription factor formation like NFATc1, cFos, PU1 and MITF causing cell fusion, cytoskeleton restructuring, Cathepsin K secretion, bone resorption etc.

Thus, it is important to note that RANKL is an important chemical mediator that is constitutively released by resident cells like osteoblasts, fibroblasts and osteocytes when bone resorption is physiologically mediated, but when the inflammatory cells release RANKL like T cells, B cells and neutrophils it might be under the influence of pro inflammatory cytokines lilke IL-1 or PGE2 which leads to pathological bone resorption casusing various signs of periodontitis. So, for a better understanding of the pathology of involvement of RANK and its ligand along with the various intracellular effector molecules which can be the target for certain therapeutic systems we have compiled this review and in conclusion we can

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