RANKL Signaling Pathway: A Potential Target for Antiresorptive Therapy in Rheumatoid Arthritis.

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic polyarthritic autoimmune condition characterized by severe bone erosion. Osteoclasts, the bone resorbing cells, are overly produced from the synovial inflammatory tissues in RA leading to excessive bone loss. RANKL-signaling pathway has been established to be the major pathway involved in osteoclastogenesis. This review highlights the role of around 40 osteoclastogenic factors involved in RANKL signaling and their potential to be targeted for antiresorptive therapy in RA. Furthermore, inhibitors of these proteins, which are already known to exhibit antiresorptive potential, have been reviewed. Elucidating new potential candidate therapeutic targets in the osteoclastogenesis pathway will open new avenues into the treatment and diagnosis of the arthritic conditions.

Keywords: RANK-RANKL signaling pathway, osteoclastogenesis, Rheumatoid arthritis, bone resorption

1. INTRODUCTION

Rheumatoid arthritis (RA), affecting about 1% of the world population, is a chronic polyarthritic autoimmune condition characterized by colossal joint destruction as a consequence of synovial hyperplasia (McInnes et al., 2011, Baron et al., 1989, Harris et al., 1990). Research studies of rheumatoid synoviocytes conducted to gain insights into the pathogenesis of bone erosion have led to the unraveling of their altered phenotype in RA patients (Davis et al., 2003). Bone and articular cartilage are invaded by pannus which is a fibrovascular structure formed as a result of proliferation of synoviocytes(Fuchs et al., 1989). Radiographic exams reveal that the symptoms of joint erosion in RA arise in very early stages and exacerbate with the advancement of the disease causing acute structural and functional impairment (Fuchs et al., 1989, van der Heijde et al., 1992). On that account, obstructing the process of bone erosion in RA becomes the most challenging and indispensible target to achieve. Extensive research indicates that ostoeclasts are the primary cells involved in the joint destruction in RA.

Osteoclastogenesis takes place at a momentously accelerated rate in Rheumatoid arthritis at a site at exterior to the marrow cavity, thereby leading to bone loss implicated in RA (Bromley et al., 1984). Consequently, abnormal bone resorption is the major pathology resulting in the functional impairment caused by rheumatoid arthritis (RA)(Bromley et al., 1984). It is supported by a multitude of research findings which suggest that synovial inflammatory tissue acts as a critical source of osteoclasts in RA by inducing localized osteoclastogenesis leading to loss in the bone mass (Fujikawa et al., 1996, Kotake et al., 1996, Takayanagi et al., 1997, Gravallese et al., 1998). Hence, overproduction of osteoclasts in RA suggests a significant connection between joint inflammation and structural damage (Schett et al., 2007). Identifying the molecular potential therapeutic targets in the osteoclastogenesis will open new avenues into the treatment and diagnosis of the arthritic conditions like RA.

Bone biology field has been greatly revolutionized since the unraveling of RANK-RANKL signaling pathway as the main pathway involved in osteoclastogenesis (Shigeyama et al., 2000). In this review, we seek to review various osteoclastogenic factors involved in RANKL signaling which have an implicated role in bone erosion associated with RA. We review therapeutic potential of thefactorswhich can be targeted to reduce bone resorption in RA.Moreover, we discuss about known inhibitors for these proteins which exhibit antiresorptive potential. Osteoclastogenic factors belonging to RANKL signaling pathway which have been reported in rheumatoid arthritis have been listed in the table. RANKL signaling pathway and osteoclastogenic factors involved in it have been demonstrated in the figure.

1.1 Bone Homeostasis

Bones are rigid yet dynamic endoskeletal organs which undergo remodeling throughout one's life span without any alterations in the size or shape (Teitelbaum et al., 2000). Maintaining normal mass in adult skeleton requires précised poise between the bone formation and bone degradation by specific types of cells (Zvi et al., 2007). Any change in the two processes leads to alterations in bone mass leading to bone-related disorders (Shcett et al., 2007, Soysa et al., 2012, Sturge et al., 2011).

1.1.1. Osteoclasts and the Bone Resorption

Osteoclasts are highly specialized, giant, unique bone resorbing polykaryons which originate from hematopoietic stem cells (Vignery et al., 2005). Myeloid progenitors undergo differentiation to form activated, bone resorbing osteoclasts via a process entailing the fusion of up to 20 mononuclear precursor cells, also known as syncytium (Rachner et al., 2011). Bone resorbing mechanism of

osteoclasts is regulated by two fundamental instrumentaries (Teitelbaumet al., 2000). First, special enzymes responsible for bone matrix degradation, such as cathepsins and matrix metalloproteinases, which cause cleavage of matrix molecules like collagen type-I and consequent removal of the non-mineralized substances from the bone. Second, vacuolar ATPase, a proton/protein pump giving rise to acidic environs between part of the osteoclast plasma membrane which demonstates metabolic activity, the disheveled boundary and the bone surface. This acidification results in solubilization of calcium from bone matrix by the cells (Li et al., 1996, Sundquist et al., 1990, Van Hille et al., 1995, Väänänen et al., 1990, Väänänen and Horton, 1995). The two aforementioned particularities allow osteoclasts to create a resorption pit by invading the bone. This resorption cavity can later be filled up by bone forming cells, osteoblasts. Osteoclasts are only found in close proximity of bones because the mineralized tissue provides them pivotal signals for their differentiation (Schett et al., 2007). Osteoclastogenesis and bone remodeling , therefore, normally occur within the bone and bone marrow (BM), except in certain pathological bone diseases, such as rheumatoid arthritis.

1.2. Role of RANKL Signaling Pathway in Bone Erosion Associated with RA

1.2.1. RANK-RANKL

RANK (Receptor activator NF-κB) and RANKL (Receptor activator of NF-κB Ligand) are the two major components of RANKL/RANK signaling pathway. Osteoblasts express RANKL protein as a type II transmembrane proteinin a soluble form resulting from the proteolytic cleavage (Wong et al., 1997, Anderson et al., 1997). Series of research studies on mice demonstrate that RANKL is indispensible in osteoclastogenesis (Theillet al., 2002). RANKL is subsequently demonstrated to bind to its receptor RANK. RANK, a type I TNFR-related transmembrane signaling protein, constituting 2-intracellular domains, expressed on the surface of osteoclasts, is activated upon RANKL binding (Woo et al., 2002). This binding results in the differentiation of osteoclast progenitor cells into mature osteoclasts followed by their activation (Lacey et al., 2000). In addition to the maintenance of survival of the mature osteoclasts, RANKL is responsible for the induction of cytoskeletal rearrangements thereby promoting bone resorption (Burgess et al., 1999, Kong et al., 1999). Moreover, osteoclast specific genes like cathepsin K and TRAF6 are also expressed as a consequence of induction by RANKL (Takahashi et al., 2011). A study entailing the immunohistological examination of tissue biopsies from patients of RA, OA and normal subjects showed the higher levels of RANKL in RA than in patients with OA or normal subjects (Zuoning Han et al., 1998).Denusumab, a human monoclonal antibody and a potent RANKL inhibitor, has been demonstrated to alleviate osteoclast resorption thus improving the symptoms of bone erosion (Kopper, 2012). Similarly, a RANK receptor inhibitor (RRI) peptide which targets a newly identified cytoplasmic motif of RANK protein, which has been reported to have a specific involvement in OC differentiation, has also shown to exhibit antiresorptive effects (Kim et al. 2009). Hence RANKL/RANK system is a potential target for pharmacological intervention in various bone disorders characterized with bone loss.

1.2.2. TRAF6

TRAF6 belongs to Tumor Necrosis Factor Receptor superfamily which is well-known for its role in osteoclastogenesis.Since RANKL lacks any intrinsic enzymatic activity, it recruits multifunctional second messangers like TRAF family members for signal transduction (Galibert et al., 1998, Wong et al., 1999, Wong et al., 1999, Lomaga et al., 1999). TRAF6 specifically induces NF-κB activity by inducing IKK complex either via TAK1 or aPKC-mediated phosphorylation (Walsh et al., 2008). In the classical NF-κB pathway, it binds to form a complex with p62 which interacts with aPKC which causes the phosphorylation of IKK-β, the critical regulator in osteoclastogenesis and bone loss (Duran et al., 2004, Sanz et al., 2000, Sanz et al., 1999). In the alternative NF-κB pathway, however, TRAF6 forms complex with TAK1 (TGFβ-activated kinase 1) and TAB1 and TAB2 adapter proteins. Upon stimulation, TAK1 phosphorylates NIK which activates IKK resulting in NF-κB activation (Mizukami et al., 2002). JNK pathway, apart from its activation by TAK1, is also activated by recruitment of p38 and its binding to TRAF6 complex by TAB1 thereby activating it (Lee et al., 2002, Ge et al., 2002). TRAF6 has been established to be the fundamental signaling adapter molecule since the TRAF6 knockout mice phenotype resulting from two independent studies show severe osteopetrotic symptoms (Naito et al., 1999, Takayanagi et al., 2000). TRAF-6 has been reported to be up-regulated in response to elevated levels of TNFα in RA patients (Aikawa et al., 2008).Moreover, inhibition of TRAF6 by IFNγ leads to a significant reduction in osteoclastogenesis which makes it a potential candidate for drug targeting (Anderson et al., 1997, Takayanagi et al., 2000). Another decoy peptide, T6DP, specifically targeting and inhibiting TRAF6 was also shown to prevent RANKL-TRAF6 mediated osteoclastogenesis (Poblenz et al., 2007). This implies that smallmolecular inhibition of TRAF6 can also be considered for antiresorptive therapy.

1.2.3. NF-κB

Rel or NF-κB, a transcription factor complex, plays significant role in numerous biological processes and hence implicated in a number of disorders including autoimmune disorders. It is activated by RANK and activates various downstream proteins involved in osteoclastogenesis. Activation of NF-kB is an immediate molecular event that takes place via recruitment of TRAF6 by RANK in osteoclastogenesis (Franzoso et al., 1997). Among five members of the NF-kB family, p50 (NF-κB 1) and p52 (NF-κB2) together play a role in osteoclastogenesis. Phenotype of p50-/- or p52-/- mice shows no bone related abnormally. However knockout mice for both of the genes show osteopetrotic phenotype implicating the essential role of the two genes in osteoclastogenesis (Iotsova et al., 1997, Takayanagi et al., 2002). NF-κB expression levels and its DNA binding activity have been demonstrated to be augmented in RA patients (Jeehee et al., 2002). Studies show that administration of an NF-κB inhibitor results in profound

reduction in osteoclastogenesis during the initial stages of the process as compared to the later stages thereby explaining its implicated role in the activation of early-stage immediate genes downstream of RANKL (Ishida et al., 2002). An inhibitor of NF-kB, has been shown to ameliorate arthritic symptoms via down-regulation of the key regulator of osteoclastogenesis in mice with collagen induced arthritis without impinging on any upstream molecules like M-CSF and RANK (Yin-Ji et al., 2012). Similarly, a peptide, known as NBD, has been shown to selectively inhibit NF- kB with significant reduction in osteoclast mediated-bone loss (Soysa et al., 2009).

1.2.4. Calcineurin-NFATc1

Nuclear factor of activated T-cells, cytoplasmic 1, belongs to NFAT family of transcription factors, which are involved in immune response. NFATc1 is the master regulator of the osteoclastogenesis owing to its indispensable role in the differentiation of osteoclasts (Takayanagi et al., 2002, Li et al., 2004, Kubota et al., 2007). Activation of NFATc1 is mediated by calcineurin which is a specific calcium-dependent serine-threonine phosphatase which dephosphorylates NFATc1 leading to its translocation into the nucleus. Inhibition by calcineurin inhibitors like cyclosporine A and FK506 results in major blockage of osteoclastogenesisthereby relieving pain in Rheumatoid arthritis rat models (Takayanagi et al., 2005,Magari et al., 2003; de la Pompa et al., 1998). Phenotype of mice deficient in NFATc1 exhibit acute osteopetrotic symptoms (Sitara and Aliprantis, 2010). In another study, in vitro experiments on NFATc1-/- demonstrate no differentiation of stem cells precursors into osteoclasts whereas in vivo analysis of NFATc1 knockout show fatal results in the embryo implicating the crucial role of NFATc1(Rangeret al., 1998, Houet al., 2001). Phenotype of TRAF6 knockout mice exhibits impaired induction of NFATc1 implying that it is one of the major immediate targets of NF-kB during the initial stages of osteoclastogenesis (Li et al., 2004). It is corroborated by the studies which show that P50/p52-knockout mice show no induction of NFATc1 (Takatsuna et al., 2005). Expression profiling of RA patients shows augmented levels of NFATc1 (Li et al., 2012). Inhibiting effects of various compounds on NFATc1 activity have been studied including cinnamaldehyde, NGDA and obovatol (Kim et al., 2013, Tsuji-Naito et al., 2008, Yamamoto et al., 1992).

1.2.5. p62

P62 is a scaffold, adaptor, ubiquitin binding- protein encoded by SQSTM1 which modulates several critical cell functions like protein turnover, internalization of receptors in cell signaling pathways, transcription of genes as well as modulation of protein-protein interactions (Moscatet al., 2007). Besides these, its significant role has been demonstrated in TRAF6 mediatedspecific activation ofNF-kB (Geethaet al., 2002). Mutations have been observed in SQSTM1 in Paget's bone disease which is characterized by high osteoclastogenic activity and thus excessive bone resorption (Morissetteet al., 2006). P392L mutation in p62 in PBD has been shown to upregulate NFATc1, the key regulator of osteoclastogenesis (Duran, et al., 2004, Sundaramet al., 2011). Phenotype of cells transfected with this mutation exhibit augmented bone erosion (Kuriharaet al., 2007) Furthermore, phenotype of SQSTM1 knockout mice results in no activation of IKK or NF-kB, no NFATc1 production and hence no osteoclastogenesisthus conspicuously playing a crucial role in osteoclastogenesis (Duran et al, 2004). Levels of p62 also need to be elucidated in other bone-related disorders characterized by increased osteoclastogenesis like osteoarthritis and rheumatoid arthritis. Not only that p62 can be targeted for therapeutic intervention, but since it works in complex with TRAF6, competitive inhibitors for TRAF6 can be designed and tested in order to reduce osteoclastogenesis by preventing the downstream activation of NF-kB pathway.

1.2.6. NIK

NF-kB -inducing kinase (NIK) is a serine/threonine protein-kinase encoded by MAPKKK14 gene. Activated by RANKL, NIK is thecontrolling component of the alternative NF-kB pathway. It is activated in response to its phosphorylation by TAK1 which occurs via formation of a complex between TRAF6 and TGF beta-activatedkinase 1 (TAK1) and adaptor proteins TAB1 and TAB2. As a result, IKK complex is activated thereby inducing NF-kB pathway (Mizukami et al., 2002). Phenotype of NIK knockout mice exhibit resistance to RANKL-mediated osteoclastogenesis suggesting their role in bone resorption by OCs (Novacket al. 2003). NIK has been demonstrated to modulate osteoclast function in rheumatoid arthritis (Aya et al., 2005). It can hence be targeted for therapeutic intervention to treat arthritic symptoms by preventing bone destruction by OCs.

1.2.7. IKK Complex

IKK complex, responsible for NF-kB activation entails three subunits includingIKKα and IKKβ, and a regulatory subunit, IKKγ thereby contributing to the production of osteoclasts and thus the increased bone resorption in inflammatory arthritis (Rothwarf and Karin, 1999). In alternative NF-kB pathway, NF-kB activation is dependent on phosphorylation of IKKα by NIK (Senftleben, et al., 2001, Xiao, G et al., 2001) In classical NF-kb pathway, however, IKKα has been shown not to have a relevant role in osteoclastogenesis in contrast to IKKβ and IKKγ, where mice lacking IKKβ develop osteopetrotic sypmtoms (Ruocco et al., 2005). IKKβ has been shown to be the central role player in osteoclast formation and its constitutive activation can result in bone erosion (Otero et al., 2010). A multitude of small molecule inhibitors for IKK have been developed in tested in vitro and in vivo for their anti-osteoclastogenic activities. Some of these include BMS-345541 (BMS) and parthenolide (PAR) which act directly against IKKαand IKKβ respectively thus blocking their kinase activity (Hehner et al., 1999,Kwok et al., 2001, Yip et al., 2004, Burke et al., 2003, Yang et al., 2006). IKK complex thus has the potential to be targeted for therapeutic intervention for treatment of diseases involving bone erosion.

1.2.8. ITAM

In addition to RANKL, osteoclast differentiation also needs certain costimulatory signals produced byDAP12 and Fc receptor common γ (FcRγ) which belong to ITAM class of adaptors (Collin-Osdoby et al., 2001, Chow et al., 1992). The

concerted activity of ITAM and RANKL signaling leads to the activation of NTATc1, the key modulator of osteoclastogenesis (Negishi-Koga and Takayanagi, 2009). OSCAR is an osteoclast-specific, Ig-like, osteoclast associated receptor protein, expressed on pre-osteoclasts and is involved in the modulation of osteoclasts function and differentiation (Kim et al., 2005,Kim et al., 2002, Ohshima et al., 2002). It is induced by RANKL and is expressed in last phases of maturation of osteoclast precursors. OSCAR has been shown to particularly signal via FcRγ and therefore can rescue osteoclastogenesis even in the absence of DAP12. OSCAR's expression in regulated by MITF and PU.1 transciption factors (Donnenberget al., 2006). It has been reported to be expressed in monocytes of RA patients thereby regulating osteoclast differentiation and ultimately increased bone resorption (Hermanet al., 2008).Mice deficient in both DAP12 and FcRγ, however, show osteopetrotic symptoms in the absence thus implicating their critical role in bone resorption (Koga et al 2004, Mocsai et al, 2004, Paloneva J, 2003).Tyrosin residues within the ITAMs are phosphorylated by SKFs (Src family kinase, with c-src being the most dominant member being expressed in osteoclasts) which then bind to SyK leading to its activation (Pitcher and van Oers, 2003, Futterer et al., 1998, Brdicka et al., 2005).Src, involved in osteoclastogensis, is activated by RANKL. Mutations leading to deficiency in src leads to osteopetrotic symptoms thus implying its potential as a therapeutic target for RA (Kawaji et al., 1995).SyK is another tyrosin kinase which has been demonstrated to play a role in osteoclast function (Faccio et al., 2003; Mocsai et al., 2004).SyK further leads to the activation of other adaptors including BLNK and SLP-76 which cause the recruitment of RANK and PLCγ-activated Tec kinases to osteoclastogenic signaling complex thereby enabling maximal influx of calcium.Btk and Tec are the major kinases from the Tec kinase family specifically expressed in osteoclasts. Phonotype of doubly deficient mice for both of these exhibit osteopetrotic symptoms thus implicating their indispensible role in bone erosion, ITAMs are hence, candidates for therapeutic intervention (Shinohara et al., 2008).

1.2.9. PLCγ

PLCs are the family of enzymes involved in the regulation ofintracellular calcium levels and expression of NFAT. They breakdown phospholipids present in biomembranes into diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3) which activate PKC and directly augment the levels of calcium by liberating it from the endoplasmic reticulum repository respectively. PLCγ2 is a member of PLCγ family and is dependent on tyrosine residue phosphorylation for its enzymatic action. It has been demonstrated that PLCγ2 knockout mutation results in mice exhibit abnormally increased bone mass in vivo. In vitro, the osteoclast precursors result in no differentiation of OC precursors into mature osteoclasts, no NFATc1 expression , defective phosphorylation of JNK, c-Jun, and IκBα phosphorylation and ultimately impaired AP1 and NF-κB activation.Moreover, PLCγ inhibitor, U73122, has shown to prevent OC development in the culture of osteoclasts thus implicating its role in osteoclast differentiation. It has been reported that PLCγ2 undergoes complex formation with Gab2(Mao et al., 2006) Gab2 (grb-2-assocaiated binding) protein belongs to another family of adaptor molecules involved in receptor signaling which also connects RANK with various downstream pathways involved in osteoclastogenesis. This is evident from the study that Gab2 deficient mice exhibit osteopetrotic symptoms in vivo thus implicating defects in osteoclastogenesis. Thus Gab2 plays a momentous role in RANKL-mediated osteoclastogenesis and is a target for therapeutic intervention (Wada et al., 2005). The PLCγ2-Gab2 complex is essential for the phosphorylation of Gab2 and that is how it is recruited to RANK for osteoclastogenesis. So PLCγ2 modulates osteoclastogenesis via ITAMs as well as Gab2 and hence is a potential candidate for antiresorptive therapeutic intervention (Mao et al., 2006).

1.2.10. AP-1/c-fos

AP-1 is a transcription factor complex with c-Fos being an essential constituent of the AP-1 transcription factor complex activated via RANK induction (Wagner et al., 2005, Johnson et al., 1992, Teitelbaum et al., 2004). C-Fos belongs to the Fos family of transcription factors and is responsible for the induction of NFATc1, the master regulator of osteoclastogenesis. This makes c-Fosan indispensible factor for osteoclastogenesis. Complete blockage of osteoclastogenesis occurs as a result of c-Fos knockout mutations in mice thereby displaying severe osteopetrotic symptoms (Wang et al., 1992, Kim et al., 2013). Levels of AP-1 and c-fos have not only been reported to be higher in RA patients but the bone resorption process in RA is also implicated to be the ultimate result of c-fos overexpression (Zuoning et al., 1998, Kurokiet al., 1993, Kurokiet al., 1993, Kurokiet al., 1994, Miyauchi et al., 1994, Trabandt et al., 1992, Shiozawa et al., 1997). An inhibitor designed by pharmacophore modeling was shown to be able to selectively inhibit AP-1/c-fos thereby resolving the arthritic condition (Harris et al., 1990). This implies that function of these proteins is indispensible for osteoclastogenesis making them potential therapeutic targets (Takayanagiet al., 2005, Asagiriet al., 2005). An attempt to inhibit AP-1 expression at transcriptional level using a sequence specific AP-1 binding oligonucleotides in has already been shown to be successful in alleviating bone erosion symtoms in a dose-dependent, sequence specific manner (Aliprantiset al., 2008).A new drug, T-5224, has also been computationally designed to inhibit AP-1/c-fossuggesting their therapeutic potential (Miyazaki et al., 2012).

1.2.11. Cathepsin K

Cathepsin Kis a cysteine protease responsible for the destruction of protein constituents of bone matrix like osteonectin, and collagens types I and II produced by synovial fibroblasts and bone resorbing macrophages, (Goto et al., 2003). It is akey regulator of osteoclast function, Cathepsin K and therefore, has an implicated role in diseases characterized by accelerated rates of joint destruction like rheumatoid arthritis, osteoarthritis and osteoporosis (Riemanet al., 2001, Kim et al., 2005). Morever, Cathepsin K has been shown to be up-regulated in response to IL-1 which is a proinflammatory cytokine and a strong inflammatory marker of RA

(Kamolmatyakul et al., 2004).Its expression, like that of TRAP (Houet al., 2001, Matsumoto et al., 2004, Anusaksathien et al., 2001), calcitonin receptor (Houet al., 2001, Matsumoto et al., 2004, Anusaksathien et al., 2001, Goll et al., 2003) and β3 integrin genes (Matsuo et al., 2006), is also modulated by NFATc1 (Matsumoto et al., 2004, Crotti et al., 2006).Cathepsin K, levels in sera of RA patients are specifically elevated and it has been shown to be responsible for joint erosion via degradation of articular cartilage since it is localized at the sites of synovial bone erosion (Martin et al., 2005). Hence, Cathespsin K is not only a potential therapeutic target for anti-rheumatic drug intervention but may also prove to be a highly exclusive diagnostic marker for RA. A specific inhibitor of cathepsin K, Odanacatib, has recently been pharmacologically evaluated for the treatment of bone loss in osteoporosis (Stoch et al., 2013).

1.2.12. PU.1

PU.1, an ETS family transcription factor, exhibits favored expression in hematopoietic stem cells and plays a prodigious role in differentiation and formation of osteoclasts via direct interaction with MITF (Simonet al., 1998, Singh et al., 1999, Tondraviet al., 1997, Luchin et al., 2001). Phenotype of knockout mice for PU.1 exhibit osteopetrotic symptoms as a result of osteoclastogenic inhibition at initial stages of differentiation (Tondravi et al., 1997). FLS of RA patients also exhibit elevated levels of PU.1 transcription factor (Itoh and Nagatani, 2012). CC-4047, an immunomodulatory derivative of thalidomide has been shown to prevent osteoclastogeneis by imposing its inhibitory effects on PU.1 (Donnenberget al., 2006). This makes PU.1 a candidate for antiresorptive therapeutic intervention.

1.2.13. MITF

Microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper transcription factor involved in osteoclast development as a result of its induction downstream of RANKL (Lu et al., 2010). NFATc1-dependent enhancement of osteoclast differentiation has a direct correlation with the elevated levels of MITF (van der Kraan et al., 2013). Moreover, phenotype of knockout mice exhibit osteopetrotic symtoms thereby implicating its significant role. Transcriptional regulator MITF results in the OC differentiation as a result of activation by p38. In collaboration with PU.1, expression of osteoclast specific genes like TRAP, cathpsin K and OSCAR is also modulated by MITF via M-CSF and RANKL signaling downstream pathways (Manskyet al., 2002, So et al., 2003; Hu et al., 2007). Levels of MITF have been shown to be reduced in response to Tacrolimus and Cyclosporin A in RA which corroborates its potential to be considered as a target for anti-rheumatic therapy (Miyazaki et al., 2007).

1.2.14. TRAP

TRAP (Tartrate-resistant acid phosphatase), a glycosylated monomeric metalloenzyme, is an established osteoclast specific marker which degrades the collagen by producing highly reactive ROS thereby leading to bone destruction (Halleenet al., 1999). TRAP 5b is a specific indicator of bone resorption and hence possesses great diagnostic importance (Halleen et al., 2000). Total TRAP activity has been demonstrated to be elevated in sera of patients with RA (Janckila et al., 2002). Moreover, phenotype of mice which have a knockout mutation for TRAP exhibit osteopetrotic symtoms thus showing their role in osteoclast function (Hayman et al., 1998). An inorganic poly(P) chain constituting 300 phosphate residues has been recently shown to strongly bind and block the activity of TRAP resulting in no formation of resorption pit required for bone degradation (Harada et al., 2013). TRAP is therefore a potential for osteoclast specific antiresorptive therapy for Rheumatoid arthritis.

1.2.15. Carbonic Anhydrase II

Carbonic anhydrase II belongs to carbonic anhydrase family of enzymes responsible for catalysis of carbon dioxide hydration in a reversible fashion. Bone resorption by osteoclasts requires a peculiar, poorly perfused, acidic, peripheral bone milieuwhich is achieved by the vacuolar proton pump activation (Vaes, 1988, Blair et al., 1989, Vaananen et al., 1990, Sundquist et al., 1990). CAII facilitates the proton production from H2O and CO2 thereby enabling the radical acidification of the resorption space (Sly et al., 1983). Distruption in CAII gene in vivo results in no bone resorption thereby implicating its critical role in optimal bone resorption process (Hall and Kenny, 1987; Raiszet al., 1988; Hott et al., 1989, Hall et al., 1991, Gay and Mueller, 1974). It has been shown to be selectively activated downstream of RANKL (Takayanagi et al., 2002). In mouse calvaria cultures, a specific inhibitor of CAII, acetazolamide, exhibits antiresorptive activity in a dose dependent, Ca2+ dependent pH-regulated manner (Lehenkari et al., 1998). Similar to acetazolamide, celecoxib and JTE-522 also inhibit osteoclast differentiation and activity which were tested in arthritic rat model for RA and showed reduced bone resorption (Katagiri et al., 2006). Levels of CAII in Rheumatoid arthritis need to be checked since it has the potential to be therapeutically targeted.

1.2.16. SH3BP2

SH3 (domain-binding protein 2), plays its role in increased osteoclastogenesis in a multitude of ways as it results in the elevated levels of both NFATc1 and the osteoclast specific gene, TRAP. Moreover, overexpression of SH3BP2 in RAW247.6 cells lead to the increased nuclear translocation of NFATc1 and hence increased osteoclastogenesis (Steven et al., 2008). Its function is critical for the calcium influx (Chenet al., 2007). It has been demonstrated to have over abundant levels in autoimmune arthritis thereby contributing to bone erosion (Gallanti et al., 2014). It is therefore a potential target for pharmacological intervention.

1.2.17. β3 Integrin

Integrins are the heterodimer proteins which are responsible for the cell-matrix interaction. v 3, a receptor for vitronectin, which is an extracellular matrix glycoprotein, has been suggested to be of paramount importance in bone resorption as it has been found to be expressed in osteoclasts in large amounts and has been implicated to modulate the attachment of sealing zone to the bone matrix (Reinholt et al.,

1990, Nakamura et al., 1996, Holt et al., 1998). Mice lacking v 3 have been shown to exhibit osteosclerotic symptoms suggesting their role in bone mass regulation (Kevin et al., 2000). In rheumatoid arthritis, not only integrins but also their ligands like collagen, fibronectin and their degradation products have been shown to have augmented levels (Postigo et al., 1993; Lowin and Straub, 2011). Antibodies against v 3 and peptides having RGD, like kistrin and echistatin, exhibit inhibitory effects on bone resorption activity (Horton et al., 1991, Lakkakorpi et al., 1991, Fisher et al., 1993). Furthermore, two inhibitors of v 3, SC-56631 and SC-65811 have also evaluated for their antiresorptive activity and hence elucidate its potential as a target for pharmacological intervention (Carron et al., 2000).

1.2.18. CaMKIV-CREB

CaMKIVis a Ca2+/calmodulin-dependent protein kinase family. CaMKIV-CREB pathway has been reported to be crucial for the differentiation and function of osteoclasts by induction of NFATc1. Differentiation of osteoclast precurors into mature osteolclasts has been demonstrated to be abrogated by blockade by CaMK inhibitor, KN-93 as well as in CREB knockout experiments (Kojiri et al., 2006). Moreover, CaMKIV gene knockout experiments and inhibition by pharmacological agents result in decreased phosphorylation of CREB, a cellular transcription factor and hence the decrease in levels of c-fos which has a major role in the induction of NFATc1. This provides basic for new therapeutic strategies (Sato et al., 2006).

1.2.19. MAPK Cascade

In addition to NF-kB/c-fos pathway, binding of RANK to its receptor RANK also leads to the subsequent activation of MAPK pathway including extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 (Li et al., 2011). MAPK signaling in RA occurs via a three-level cascade of kinases called MAPKs. MAPKs or MAP kinases are the serine/threonine/tyrosine-specific protein kinaseswhich belong to (CDK/MAPK/GSK3/CLK) group of kinases. Involvement of MAPK cascade has been reported in rheumatic conditions (Sweeney et al., 2004; Schett et al., 2000). MAPKKK or MEKKs are the most upstream kinases which work in connection with GTPases like Rho and Ras, the latter being associated with chronic synovial inflammation (Marinissen et al., 2001).Nitrogen-containing biphosphate drugs inhibit Ras thereby showing anti resorptive effects (Luckman et al., 1998; Fisher et al., 1999). MEKKs activate downstream MAPKKs which in turn, activate p38 and JNK pathways. MKK-7 specifically activates JNK pathway while MKK-4, in some cases also lead to phosphorylated activation of p38MAPK in addition to JNK (Yan et al., 1994, Blank et al., 1996, Gerwins et al., 1997).MEKK-1 has been shown to have elevated levels in RA patients as well as in cultured synoviocytes, which also overly express MEKK-2 (Hammaker et al., 2004). Other MAPKKKs like TAK1 and MTK specifically activate p38MPAK via induction of MKK-3 and MKK-6 (Takekawa et al., 1997, Yamaguchi et al. 1995).TAK1 has been reported to have augmented levels in synovial tissues and synoviocytesin RA patients (Hammaker et al., 2004). MKK-3 and MMK-6 have been reported to specifically induce p38 whereas MEK-1 and MEK-2 are basically responsible for the activation of ERKs (Boyle et al., 2014; Fanger et al., 1997). An inhibitor of MEK-1 has shown to block differentiation of osteoclasts precursors thus implicating its role in osteoclastogenesis. Blocking of Mek ½ via a strong specific inhibitor ARRY-162 in AIA, which has entered phase-II clinical trials corroborates their therapeutic potential (Lindstrom et al., 2010). Hence, pharmacological intervention which blocks their activity or JNK knockout transfection results in repression of RANKL-mediated osteoclast formation (Ikedaet al., 2004).

MKKs, downstream to MAPKKKs, are responsible for phosphorylating p38MAPK. The most significant MKKs are MKK-3 and MKK-6 which is evident from the p38 lacking phenotype of the mice with double knockout mutation for both of these MKKs (Cong et al., 1999). MKK-3 leads to the preferential induction of p38MAPK in synovial fibroblast as a result of their exposure to TNF and IL1 (the proinflammatory cytokines) (Inoue et al., 2006, Moriguchi et al., 1996). Overexpression of p38 also leads to the increased expression of MKK-3 and MKK-6 in the patients with RA (Chabaud-Riou et al., 2004). As a result, p38 is strongly expressed in synovial membrane and in the osteoclasts at the site of synovial invasion (Hayer et al., 2005). P38 and other MAPKs also control regulation of MMPs.

Hence increased MAPK levels implicate increased MMP and thus increased collagen destruction(Liacini et al., 2003, Suzuki et al., 2000). This is corroborated by alleviation of cartilage destructive symptoms via p38 blockade (Zwerina et al., 2006) In addition; SCIO-469 another p38 inhibitor has shown to block osteoclastogenic process by preventing formation of osteoclasts (Nguyen et al., 2006). A JNK-specific inhibitor, SP600125, has also shown to inhibit anti-apoptotic characteristics of osteoclasts (Ikeda et al., 2008). Hence, the accumulating evidence suggests the therapeutic potential of MAPK cascade.

1.2.20. PPARα

PPARα, is a member of nuclear receptor family, is a transcription factor activated by ligand.There exist three subtypes of PPAR namely PPAR α β, and γ and all of them have been shown to be expressed in osteoclasts (Cernuda-Morollon et al., 2002; Mano et al., 2000; Mbalaviele et al., 2000). The levels of PPAR-α have been demonstrated to be elevated in the synovial fluid of patients with rheumatoid arthritis. A small ligand, fenofibrate designed to inhibit PPAR-α has shown to result in reduced bone erosion making it a potential target for anti-rheumatic therapy (Okamoto et al., 2005).

2. CONCLUSION

Bone disorders like rheumatoid arthritis are characterized by bone erosion which is a consequence of abnormal regulation of balance between bone formation and bone resorption. Osteoblasts and osteoclasts are responsible for maintaining proper bone mass. Since there has been accumulating evidence suggesting the increase in osteoclastogenesis in RA, joint destruction is considered as the major pathology associated with RA. Although exact etiology of RA remains

obscure, several genetic factors have been found to be associated with osteoclastogenesis which play their role in differentiation, formation, activation, cell-cell fusion and survival of osteoclasts thereby enabling excessive bone erosion. These mainly constitute the set of genes playing their roles in RANKL signaling pathway including NFATc1, the master regulator of osteoclastogenesis. RANKL signaling pathway activates multiple downstream pathways including MAPKs (p38, ERK and JNK), classical and alternative NF-κB pathways and CamKIV-CREB all of which eventually result in the AP-1/c-fos/NFATc1 induction leading to stimulation of osteoclastogenic pathway. Expression profiles of the patients with bone related disorders like osteoporosis, osteoarthritis and rheumatoid arthritis have shown to exhibit augmented levels of these osteoclastogenic factors and hence the excessive bone degradation. Natural and synthetic inhibitors against some of these factors have already been evaluated from pharmacological standpoint and some of them have shown their anti-arthritic activity by blocking osteoclastogenesis. For example,targeting NFATc1 by inhibitors like obovatol and cinnamaldehyde inRA has shown antiresorptive effects. Hence, using derivatives of already used compounds can therefore help elucidate more potent actives. In addition, expression levels of certain proteins activated downstream of RANKL signaling pathway like carbonic anhydrase II need to be elucidated in Rheumatoid arthritis and osteoarthritis and can be targeted for pharmacological intervention.

Table 1. List of osteoclastogenic factors involved in RANKL signaling reported in RA and their inhibitors

Protein	Family	Inhibitors	References
Ras	GTPase	N-BP drugs	Marinissen et al., 2001 Luckman et al., 1998 Fisher et al., 1999
MEK 1/2	MAPKK	ARRY-162	Wright et al. Lindstrom et al., 2010
TAK1	serine/threonine protein kinase family	_____	Hammaker et al., 2004
P38	MAPK	SCIO-469	Hayer et al., 2005 Nguyen et al., 2006 Inoue et al., 2006 Moriguchi et al., 1996
JNK	MAPK	SP600125	Ikeda et al., 2008
MKK-3	MAPKK	_____	Chabaud-Riou et al., 2004 Cong et al., 1999
PPARα	nuclear receptor family	Fenofibrate	Okamoto et al., 2005
MKK-6	MAPKK	_____	Chabaud-Riou et al., 2004 Cong et al., 1999
αvβ3	Integrins	Kistrinechistatin SC-5663 SC-65811	Postigo et al., 1993 Lowin and Straub, 2011 Carron et al., 2000
TRAP	glycosylated monomeric metalloenzyme	inorganic poly(P) chain	Harada et al., 2013
MITF	helix-loop-helix leucine zipper transcription factor	TacrolimusCyclosporin A	Miyazaki et al., 2007
PU.1	ETS family transcription factor	CC-4047	Itoh and Nagatani, 2012 Donnenberget al., 2006
Cathepsin K	cysteine protease	Odanacatib	Martin et al., 2005 Stoch et al., 2013

AP-1/c-Fos	Transcription factor	AP-1 binding oligonucleotides T-5224	Zuoning et al., 1998 Kurokiet al., 1993 Kurokiet al., 1994, Miyauchi et al., 1994, Trabandt et al., 1992, Shiozawa et al., 1997 Aliprantiset al., 2008 Miyazaki et al., 2012
OSCAR	osteoclast-associated receptor	_____	Hermanet al., 2008
NIK	serine/threonine protein-kinase	_____	Aya et al., 2005
Calcineurin	calcium-dependent serine-threonine phosphatase	cyclosporine A FK506	Takayanagi et al., 2005 Magari et al., 2003 de la Pompa et al., 1998
IKK complex		BMS-345541 (IKKα) parthenolide (IKKβ)	Rothwarf and Karin, 1999 Hehner et al., 1999 Kwok et al., 2001 Yip et al., 2004 Burke et al., 2003 Yang et al., 2006
TRAF6	Tumor Necrosis Factor Receptor superfamily	IFNγ T6DP	Aikawa et al., 2008 Anderson et al., 1997 Takayanagi et al., 2000 Poblenz et al., 2007
RANK	*type I TNFR-related transmembrane signaling protein*	*RANK receptor inhibitor (RRI) peptide*	*Kim et al. 2009*
RANKL	*type II transmembrane protein*	*Denusumab*	*Zuoning Han et al., 1998* *Kopper, 2012*
Carbonic anhydrase II	*Carbonic anhydrase*	*acetazolamidecelecoxib* *JTE-522*	*Lehenkari et al., 1998* *Katagiri et al., 2006*
SH3BP2	*SH3 domain-binding protein*	_____	*Steven et al., 2008* *Gallanti et al., 2014*

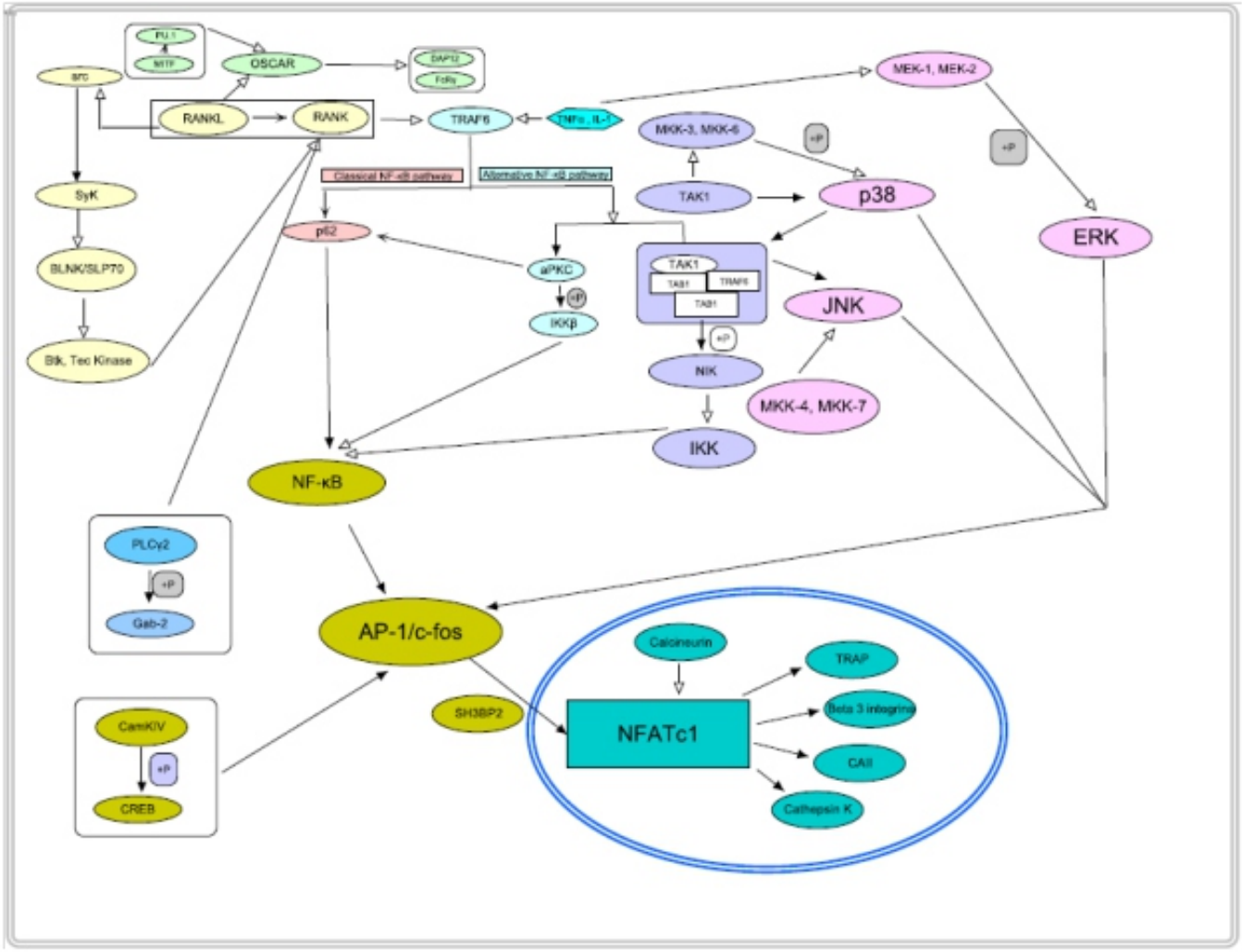

Figure 1. Osteoclastogenic Factors in RANKL Pathway

Binding of RANKL to its receptor RANK leads to the activation of TRAF6 thereby inducing downstream classicial and alternative NF-κB pathways. Classical pathway is induced via induction of p62 by aPKC. The alternative NF-κB pathway, is activated either via phosphorylation of IKKβ by aPKC or by phosphorylation of IKK complex by NIK as a result of TRAF6/TAK1/TAB1/TAB2 complex formation. This also results in the activation of MAPKs like p38, ERK and JNK via MEKKs and MEKs which induce AP-1/c-fos. In addition to its activation by NF-kB, AP-1/c-fos is also activated by CREB after its phosphorylation by CamKIV. AP-1/c-fos induction leads to the activation of NFATc1 while SH3BP2 plays its role in its nuclear translocation. Activation of NFATc1 leads to the downstream expression of various osteoclast specific genes like Cathepsin K, carbonic anhydrase II, TRAP and β3 integrins. RANKL also induces downstream activation of src and OSCAR which lead to activation of Tec Kinases and ITAMs like DAP12 and FcRγ respectively, all essential for osteoclastogenesis. PLCγ2 forming complex with gab2 thereby phosphorylating it leads to the recruitment to RANK to its osteoclastogenic signaling complex.

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