The RANKL-RANK Story

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Key Words

RANK · RANKL · OPG · Estrogen · Osteoporosis · Cancer · Thermoregulation · Immunity · Osteoclastogenesis

Abstract

Receptor activator of nuclear factor κ B (RANK) and its ligand (RANKL) have originally been described for their key roles in bone metabolism and the immune system. Subsequently, it has been shown that the RANKL-RANK system is critical in the formation of mammary epithelia in lactating females and the thermoregulation of the central nervous system. RANKL and RANK are under the tight control of the female sex hormones estradiol and progesterone. A reduction of the circulating female sex hormones leading to an increase in RANKL-RANK signaling is the leading cause of osteoporosis in postmenopausal women. Denosumab, a human monoclonal anti-RANKL antibody, has been approved for the treatment of postmenopausal osteoporosis, where it is showing great promise. In addition, RANKL-RANK signaling also plays a critical role in other bone pathologies, bone metastasis or hormone-driven breast cancer. This review will highlight some of the functions of RANKL-RANK in bone turnover, the immune system and brain with a focus on the regulatory role of the female sex hormones.

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Introduction

Bone remodeling and metabolism from early development and throughout adulthood are performed by the orchestrated action of osteoblasts, which produce new bone, and osteoclasts, which break it down. Osteoblasts are mononuclear terminally differentiated cells that arise from a mesenchymal stem cell (MSC) lineage [1]. Osteoclasts, originally described in 1873 by Albert Kolliker, are large multinucleated cells that stem from a hematopoietic lineage by the fusion of mononuclear osteoclast progenitor cells [2]. Tightly regulated bone turnover depends on the delicate balance of these two cellular systems whose deregulation, caused by hormonal, inflammatory or growth factor changes, leads to several life-altering diseases including osteoporosis (loss of bone mass) and osteopetrosis (gain of bone mass). Understanding the molecular mechanism of the regulation of these cellular systems is therefore critical in addressing these major health concerns.

The spark initiating the search for factors contributing to osteoclastogenesis was ignited by the hypothesis Rodan and Martin [3] put forth as early as 1981, postulating that osteoblasts influence the formation of osteoclasts. In the late 1990s, it was discovered that osteoclastogenesis can be induced by the ligand of receptor activator of nuclear factor κ B (RANKL) and its receptor RANK, whereas osteoprotegerin (OPG) acts as a soluble molecular decoy [4–7]. Around the same time, it was reported that RANKL also resides on the surface of activated T-cell

lymphocytes, where it has been suggested to function in regulating adaptive immunity [8, 9]. In addition, RANKL-expressing T cells can influence osteoclastogenesis, explaining bone loss in diseases with chronically active inflammatory responses [10]. The final clarification of the role RANKL plays in immunity and bone metabolism came from mutant mice lacking RANKL, which exhibited complete defects in osteoclastogenesis resulting in severe osteopetrosis and a failure in tooth eruption as well as impaired lymph node formation [11]. The recognition of this dual function of RANKL in immunity and bone homeostasis seeded the rapidly growing field dubbed osteoimmunology [12].

However, the multifaceted role of this molecular triad is not limited to these functions. Recent studies have begun revealing a role for the RANKL/RANK/OPG system in distinct areas of the central nervous system (CNS) where it participates in thermoregulation and plays a protective role in ischemic stroke [13, 14]. Intriguingly, the expression and function of the RANKL/RANK/OPG axis is strongly influenced by the female sex hormones, underlining the gender bias of this complex molecular system. While some of its functions are shared by males and females, others are specific for female physiology and will be discussed here.

RANKL, RANK, OPG and Bones

The tumor necrosis factor superfamily 11 (TNFSF11) gene encoding RANKL is also known as tumor necrosis ligand superfamily member 11, TNF-related activation-induced cytokine, OPG ligand or osteoclast differentiation factor. RANKL is a homotrimeric type II transmembrane protein which can be released from the cell membrane by proteolysis by one of several extracellular proteases including a disintegrin and metalloprotease and metalloprotease-7 [15, 16]. RANKL was first identified on the surface of osteoblasts which participate in multiple stages of osteoclastogenesis and bone metastasis [4–7]. Two receptors have been reported for RANKL, the membrane-bound receptor RANK, also known as TNF-related activation-induced cytokine receptor or TNF receptor superfamily member 11A (TNFRSF11A), and the soluble decoy receptor OPG or TNFRSF11B.

Prior to the discovery of RANKL, it was known that the macrophage colony-stimulating factor (M-CSF) is necessary for blocking apoptosis of osteoclast progenitors and is thus required for osteoclast development [4]. M-CSF supports the proliferation and cell survival of osteo-

clast precursors by binding its receptor, c-Fms, and recruiting the growth factor receptor-bound protein 2, phosphatidylinositol 3K/Akt and phospholipase C γ (PLC γ) pathways [17]. Indeed, M-CSF mutant mice develop osteoporosis due to a lack of macrophages and osteoclasts [18]. Together with RANKL, these two cytokines are sufficient and necessary factors for osteoblasts to trigger osteoclast precursors to differentiate into functional osteoclasts: M-CSF acts as a survival factor for the early progenitors, whereas RANKL-RANK provides the key instructive osteoclast lineage signal. In addition, RANKL and RANK are present in many tissues including the lymph nodes, thymus, spleen, brain and bone marrow [19]. In bone, RANKL is expressed in stromal cells, osteoblasts and their precursors, while RANK is present on the cell surface membranes of osteoclasts and their progenitors. Recent evidence has shown that osteocytes are also an abundant source of RANKL in activating osteoclastogenesis; the specific deletion of RANKL in mouse osteocytes results in an increase in bone volume and mass, the significant absence of active osteoclasts and severe osteopetrosis [20].

Binding of either the soluble or the membrane-bound RANKL to RANK causes receptor oligomerization, and the recruitment of one of several adaptor molecules, including TNF receptor-associated factor 6 (TRAF6), to specific sites on the membrane-proximal portion of RANK [21] (fig. 1). That TRAF6 is an essential component of RANKL-RANK signaling in the formation of osteoclasts, and not other members of the TRAF family that also bind TNF receptors, is underscored by reports that only TRAF6 mutant mice exhibit severe osteopetrosis [22, 23]. The recruitment of TRAF6 leads to the activation of several signaling pathways including NF- κ B, mitogen-activated protein kinase family and/or c-Src resulting in osteoclastogenesis, survival or the activation of their resorptive activities [17] (fig. 1). The recruitment of the mitogen-activated protein kinase family leads to the nuclear translocation of transcription factors c-Fos and c-Jun [24]. NFATc1 requires the release of intracellular Ca²⁺ by PLC γ for activation. Both M-CSF and RANKL activate PLC γ via immunoreceptor tyrosine-based activation motif-containing molecules [17]. The activation of NF- κ B and subsequent upregulation of c-Fos are critical steps in osteoclastogenesis, demonstrated by mutant mice lacking NF- κ B subunits p50 and p52 or c-Fos, which develop osteoporosis due to a lack of osteoclasts [25, 26]. Importantly, together c-Fos and NFATc1 trigger the transcription of the genetic program required for osteoclastogenesis. TRAF6 can also form a complex with c-Src to activate

Fig. 1. RANKL-RANK-mediated osteoclastogenesis and signaling. **a** Stimulated by parathyroid hormone, vitamin D and/or prostaglandin 2 (and many other factors not shown here), osteoblasts and osteocytes present RANKL to osteoclast progenitor cells. OPG inhibits RANK signaling by preventing RANKL-RANK interactions. E2 upregulates OPG and thus interferes with RANKL-RANK activation. E2 can also extend or limit osteoblasts and their life span. Progesterone is a key inducer for RANKL in vivo (not shown here). **b** Upon binding to RANKL, the RANK receptor undergoes trimerization and recruits the TRAF6 adaptor molecule which couples RANK to several signaling pathways. c-Src activates

an anti-apoptotic program via Akt/protein kinase B. The activation of mitogen-activated protein kinase induces the translocation of c-Fos and c-Jun into the nucleus. The translocation of NF-κB into the nucleus triggers the upregulation of c-Fos, which in a complex with Ca^{2+} -regulated NFATc1 initiates a genetic program required for the formation of mature osteoclasts. Many other signaling molecules involved in this pathway have been omitted as they are beyond the scope of this review and are reviewed elsewhere [17]. PTH = Parathyroid hormone; PGE2 = prostaglandin 2; NFATc1 = nuclear factor of activated T cells c1.

the anti-apoptotic serine/threonine kinase, Akt/protein kinase B resulting in the phosphorylation and inhibition of the pro-apoptotic protein Bad [27]. Following multinucleation by the fusion of mononucleated osteoclast precursors, RANKL initiates the cytoskeletal reorganization and cellular polarization of mature osteoblasts to trigger their bone-resorbing activity [17].

OPG is produced as a soluble decoy receptor by MSCs, osteoblast/stromal cells and various other cell types, and it has been shown to inhibit RANKL-RANK signaling [4, 6, 7]. A partial deletion of gene TNFRSF11B, encoding for OPG on chromosome 8q24.2, leads to juvenile Paget's disease due to a significant increase in RANKL-RANK interactions [28]. Paget's disease is marked by rapidly in-

creased bone remodeling, skeletal deformities, osteopenia and bone fractures. In contrast to mice lacking RANKL or RANK, OPG mutant mice develop severe osteoporosis due to increased numbers and activity of osteoclasts [29, 30]. OPG inhibits osteoclastogenesis and the activation of osteoclasts and therefore provides the delicate balance of bone turnover.

RANKL, RANK and OPG in Immunity

It has been reported that RANKL and RANK are not only expressed on cells of the immune system, in particular B cells and activated T lymphocytes, but that they also participate in their maturation and survival [8, 9]. For instance, human patients with mutations within the RANK gene *TNFRSF11A* have several defects in B-cell immunology [31]. Moreover, mice carrying a germline deletion of *RANK* or *RANKL* have altered B-cell development, resulting in significantly reduced numbers of B cells [11, 32]. However, mice lacking RANK specifically in their B-cell populations have normal numbers of pro-, pre- and immature B cells in the bone marrow [33]. Furthermore, basic B-cell functions required for humoral immunity are normal in these mice. It can therefore be concluded that the B-cell defects in human patients and *RANKL* and *RANK* full body knock-out mice are secondary and can be attributed to the lack of bone marrow cavities by the increased bone mass in osteopetrotic bones [33]. For this reason, the use of a *RANKL* blockade in adults with osteoporosis (discussed in more detail below) does not affect normal B-cell physiology as originally proposed. Nevertheless, B cells can be a potent source of RANKL, especially in the case of periodontal lesions or multiple myelomas (MM), where they contribute to bone loss by regulating the activity of osteoclasts [34, 35].

In the thymus, RANK signaling is required for the development of a particular type of thymic epithelial cells, which express the autoimmune regulator and are implicated in T-cell self-tolerance [36, 37]. *RANK* and *RANKL* knock-out animals display a complete lack of CD80⁺ AIRE⁺ medullary thymic epithelial cells. Thus, the induction of central T-cell tolerance appears to be affected, and such mice indeed develop mild autoimmunity at an older age. Moreover, RANK signaling was shown to increase T-cell viability *in vitro* [9]. *RANKL* and *RANK* are also expressed on dendritic cells (DCs) which present antigens to T cells [9]. An interaction of *RANKL* from activated T cells and *RANK* on DCs results in the upregulation of anti-apoptotic molecules leading to increased numbers and persis-

tence of antigen-presenting DCs, increased antigen-specific T-cell and enhanced memory T-cell responses [9].

In addition, *RANKL* and *RANK* knock-out animals have no lymph nodes but apparently an intact splenic architecture and Peyer's patches [11, 32]. Lymph nodes are a critical component of the lymphatic system, where lymphocytes are concentrated and honed. It has been suggested that the absence of lymph nodes in *RANKL* or *RANK* knock-out mice can be attributed to the defective development of lymph node-forming cells, although the exact role of *RANKL*-*RANK* in the organogenesis of lymph nodes needs to be elucidated. The development of lymph nodes might depend on PLC γ 2, which when absent in mice causes a similar lack of lymph nodes as in *RANKL* and *RANK* mutant mice. As a matter of fact *RANKL*-*RANK* signaling is impaired in PLC γ 2 mutant animals, causing a failure for bone marrow progenitors to develop into osteoclasts [38]. Taken together, *RANKL* and *RANK* appear to play multiple roles in the development of immunity-regulating T cells in the thymus via AIRE⁺ medullary thymic epithelial cells and lymph node organogenesis, and they possibly have a role in functional T-cell-DC interactions. The expression of *RANKL* in cells of the immune system is believed to contribute to the pathogenesis of several autoimmune diseases such as rheumatoid arthritis, where inflammatory signals are coupled to the destruction of bones and joints via *RANKL* production [10].

Sex Hormone Regulation of RANKL and OPG

The expressions of *RANKL*, *RANK* and *OPG* are regulated by a variety of different hormones and cytokines promoting or blocking the formation of osteoclasts at different stages of the development including the parathyroid hormone and its related protein, steroid hormone 1,25(OH) $_2$ D $_3$, prostaglandin, TNF- α , interleukin 6 (IL6) and M-CSF [39–42] (fig. 1). The female sex hormone estradiol (E2) regulates the levels of many of these factors: the loss of E2 during menopause causes severe bone loss leading to bone fractures and a deformation of the skeleton. Hormone replacement therapy (HRT), containing a combination of E2 and progesterone or E2 alone, has shown to be effective in inhibiting the activity of osteoclasts and restoring bone mass [43]. The loss of circulating E2 results in a marked upregulation of proinflammatory cytokines IL1, IL6 and TNF- α , and a significant decrease in TNF- β , the cumulative effect of which causes increased osteoclastic activities and subsequent bone loss

Fig. 2. The role of estrogens in bone remodeling. High levels of E2 result in high TGF- β and high OPG expressions. OPG inhibits RANKL signaling and promotes bone formation. Conversely, when circulating E2 levels drop during menopause or after OVX in female rodents, inflammatory cytokine levels increase, including IL1 and IL6 levels. TNF- α is increased by T cells, resulting in increased levels of RANKL and consequently an increased activity of osteoclasts. TGF- β = Transforming growth factor β .

[43] (fig. 2). In addition, increased IL6 is also regarded as a potent tumor-promoting factor in various types of human cancers including glioma, lymphoma, melanoma as well as breast, ovarian, pancreatic, prostate, renal and colorectal cancer [44]. IL6 is also strongly produced by stem-like breast cancer cells (CD44⁺/CD24^{low}) in triple negative breast cancer [45]. Furthermore, E2 can block bone-resorbing activity by blocking these proinflammatory cytokines from stromal cells, monocytes and lymphoid cells [46, 47]. By modulating the balance of these cytokines, E2 can modulate the opposing activities of OPG and RANKL. In human osteoblasts, E2 influences osteoclastogenesis by directly increasing the level of OPG and to a lesser extent that of RANKL mRNA and protein [48, 49]. E2 receptors α and β are expressed in stromal cells, osteoblasts, osteoclasts and their progenitors, and they can mediate E2 effects on these cells directly [50]. For instance, E2 mediates the life span of osteoblasts and osteoclasts [51]. E2 upregulates osteoblast production of transforming growth factor- β which limits the life span of osteoclasts and induces apoptosis in a mixed cell culture [52]. The proapoptotic effects of E2 on osteoclasts can also be mediated directly in osteoclasts by the activation of E2 receptor α , ultimately resulting in a shortened cellular life span and the inhibition of their bone-resorbing activity [53]. It is likely that these effects are mediated by nongenotropic activities of E2 receptors via Src/Shc/ERK signaling [51]. Further, it has been shown that ovariectomy (OVX)-induced bone loss in mice can be, at least in part, attributed to the expansion of TNF- α -producing T

cells and reversed by E2 [54]. Moreover, an increased number of T cells, induced by a loss of E2, has been attributed to interferon- γ which inhibits RANKL-RANK signaling by degrading TRAF6 [55].

Female sex hormones regulate the development of mammary epithelia during pregnancy and interestingly they do so via the RANKL/RANK/OPG axis [56]. This molecular connection was discovered by the unexpected observation that RANKL mutant female mice were not lactating, and their pups were dying soon after birth [56]. Proper maturation of the functional mammary gland is driven by pregnancy-related female hormones such as E2, progesterone and prolactin, and the calcium-rich milk is produced by osteoclast-regulated calcium mobilization [57]. RANKL mutant animals exhibit a complete defect in lobuloalveolar epithelial development in pregnancy and consequently an absence of lactating mammary glands. Later detailed studies identified progesterone as the key hormone to regulate RANKL in mammary epithelial expansion in pregnancy and provided the basis for targeting RANKL during progesterone-driven breast cancer (see below) [58–63]. Moreover, through RANKL regulation, progesterone increases the proliferation of Lin-CD24⁺CD49f^{hi} mammary stem cells also found in mammary tumors [63]. These findings were not only entirely unexpected in mammary gland biology, but also underscored the importance of the RANKL/RANK/OPG axis in females, not just in the context of bone metabolism and immunity but also during pregnancy in preparation for lactation.

Fig. 3. The role of RANK-RANKL in CNS. **a** Transient ischemia induces RANK-RANKL and OPG expression in macrophages and microglia of the mouse cortex. This increase causes a downregulation of inflammation-promoting factors such as IL6 and TNF- α . **b** Various pyrogens, such as the inflammatory factors via IL-1 β or TNF- α lipopolysaccharide, increase RANKL expression in the hypothalamus, resulting in RANK signaling in the glia. RANK signaling initiates a febrile response via prostaglandin 2/prostaglandin E receptor 3. LPS = Lipopolysaccharide; PGE2 = prostaglandin 2; EP3 = prostaglandin E receptor 3.

RANKL, RANK, OPG and the CNS

While the role of the RANKL/RANK/OPG axis in bone remodeling has been extensively studied for well over a decade, the role of these molecules in the CNS has only begun to emerge. RANKL mRNA was localized to the brain; nevertheless, the roles of RANK-RANKL and OPG have only recently been reported in discrete regions of the hypothalamus and in the ischemic cortex [5, 64]. Our group has localized RANK and RANKL expression to the areas of the hypothalamus involved in the regulation of the febrile response, namely the preoptic area, and the medial and lateral septal nuclei [13] (fig. 3). Generally, fever, thought to be an important component of the immune response, is triggered by endogenous or exogenous pyrogens such as proinflammatory cytokines, including IL1 β , IL6 and TNF- α or lipopolysaccharide. These triggers result in the cyclooxygenase or phospholipase 2-mediated release of prostaglandin E2, which acts on the prostaglandin E receptor 3 in the hypothalamus to increase body temperature [65]. Injections of RANKL directly to the lateral ventricles of the brain induced a marked and rapid increase in core body temperature in

both mice and rats. No such temperature increase was noted in CNS-specific conditional RANK knock-out mice. Further, although RANK is expressed in neurons, microglia and astrocytes, we reported that only glial fibrillary acidic protein-positive glial cells mediate this cyclooxygenase 2-dependent fever response. In addition, patients with a homozygous Arg170Gly mutation in the RANK-coding gene had an abrogated febrile response to pneumonia as compared to age-matched controls, corroborating the animal model data [13]. Interestingly, in these studies, a gender-specific effect was observed. We found that CNS-specific RANK mutant female mice had an elevated resting body temperature compared to control littermates. Moreover, the loss of ovarian sex hormones induced by OVX resulted in a decrease of RANKL mRNA in the brain and an increase of core body temperature in control animals but not in CNS-specific RANK knock-out females [13]. Serum RANKL fluctuates in response to the adult female estrus cycle in mice and is markedly increased with OVX. It is not clear yet how peripheral and CNS levels of RANKL in response to sex hormones are correlated. Nevertheless, these results provided the first evidence of RANKL-RANK function in the CNS and showed that this system is a critical component of the central febrile response. Moreover, it appears that the link between sex hormones and female temperature homeostasis is mediated by the RANKL-RANK system.

Recently, another unexpected role for the RANKL/RANK/OPG axis has been reported in the CNS, one independent of temperature regulation [14]. Shimamura et al. [14] highlighted an anti-inflammatory and neuroprotective role of the RANKL-RANK system in ischemic stroke. Middle cerebral artery occlusion, a model of transient ischemia, induced the mRNA expression of RANKL-RANK and OPG in brain, and increased protein levels of the three molecules in the ischemic cortex within activated microglia and macrophages in mice (fig. 2). They further reported that OPG mutant mice with elevated levels of RANKL in the middle cerebral artery occlusion model had a reduced infarct volume and decreased cerebral edema compared to control littermates. Likewise, intraventricular injections of active RANKL 4 h after middle cerebral artery occlusion resulted in a similar reduction of the infarct volume, a reduced cerebral edema and also reduced mortality rates. These effects are most likely mediated by the downregulation of the microglial inflammatory cytokines IL6 and TNF- α through RANKL [14] (fig. 2). An interesting question that arises from these studies is whether there is a gender bias here as well: is this the molecular explanation why more women than men

have strokes? Nevertheless, these findings uncover an exciting novel beneficial role of the RANKL/RANK/OPG axis and suggest that we have only just begun to understand the complexity of this molecular triad.

RANKL/RANK/OPG-Based Therapies

Due to the wide range of functions, abnormalities in the RANKL/RANK/OPG system lead to an extensive range of diseases from postmenopausal osteoporosis, Paget's disease, rheumatoid arthritis, to breast cancer [11, 28, 63, 66]. Increased RANKL-stimulated osteoclast activity initiated by the loss of E2 at the onset of menopause in many cases causes osteoporosis, marked by a progressive decrease in bone mass. HRTs have been widely used to inhibit bone loss and protect against heart disease and several types of cancers. However, a major study by the Women's Health Initiative and the Million Women Study reported that HRT increased the incidence of breast cancer amongst other side effects [67, 68]. For this reason, more specific therapeutic targets are necessary for the treatment of osteoporosis. Denosumab, a fully human monoclonal antibody raised against RANKL, blocks its binding to and the activation of RANK and thereby inhibits RANK-mediated osteoclastogenesis leading to an increase in bone mass [69]. Blocking RANKL-RANK activity on osteoclasts is also effective against bone loss associated with bone metastases and skeletal-related events (SREs) in solid tumors, prostate cancer or breast cancer. However, in randomized trials of bone metastasis from either breast or prostate cancers, while denosumab significantly improved SREs as compared to zoledronic acid therapy, there was no improvement in survival benefit [70, 71]. These studies were expanded to include bone metastasis from a wide range of cancers, including MM [72], and again denosumab proved more beneficial than zoledronic acid therapy in several aspects including SREs as well as a slight improvement in survival benefit except in the case of MM where more favorable survival was demonstrated by patients receiving zoledronic acid [72]. MM is a malignant disease marked by the accumulation of plasma cells in the bone marrow, the presence of osteolytic lesions resulting in SREs, bone pain and osteopenia stemming from a multicellular dysregulation of the RANKL/RANK/OPG axis especially in MSCs. Targeting these differentiation-defective MSCs in MM is a focus of great interest in finding alternative therapeutics for MM [73].

In addition, the inhibition of RANKL-RANK has shown tremendous clinical efficacy in giant cell tumors

[74]. RANKL expression was also observed in human breast cancer cells, having little effect on proliferation and survival, but causing a marked increase in migration and bone tumor metastasis [66]. Moreover, our group was able to demonstrate that synthetic progestin, medroxyprogesterone, used in HRT, increased RANKL expression in mammary epithelium [63]. In turn, this triggered massive Lin-CD24⁺CD49f^{hi} mammary stem cell proliferation and protection against DNA-damage-induced apoptosis [63, 75]. Indeed, we and others also showed that progesterone-driven mammary cancer is regulated by RANKL [63, 76]. These effects were abrogated by blocking RANK signaling using mammary-specific RANK mutant animals as well as by blocking RANKL using RANK-Fc, suggesting that RANKL-RANK signaling is necessary for progestin-driven mammary tumors and that RANKL-RANK inhibition provides a novel therapeutic target in the fight against breast cancer [63, 76]. Considering the diverse function of RANKL/RANK/OPG in human physiology, the inhibition of denosumab causes a remarkably low incidence of extraskelatal side effects. It is worth mentioning that skin irritations such as eczema and cellulitis have been reported with denosumab treatment [77]. RANKL expressed in keratinocytes of inflamed skin mediates the function of local regulatory T cells, thereby inhibiting RANKL activity with denosumab might interfere with immunity of the skin [78]. However, even OPG knock-out animals, where RANKL levels are chronically elevated, have no apparent immune defects [79]. In addition, a dose-dependent increase in the incidence of mild hypocalcemia has been reported with the administration of denosumab; however, overall, denosumab has been reported to be superior to other therapies in increasing bone density in osteoporosis and preventing SREs [77].

Conclusions

RANKL/RANK/OPG play key roles in the development of osteoclasts and the regulation of the immune system. While we have begun to grasp their central role in osteoimmunology in the last decade, the emerging data for their function in the CNS is only in its infancy. Here we reported that RANKL-RANK is necessary for regulating the febrile response to infections and possibly during the fluctuations of female sex hormones. As many women report having sudden bursts of temperature increase, or 'hot flashes', it is of interest whether RANKL-RANK signaling is underlying this phenomenon during menopause. An increase in RANKL in OPG mutant animals

plays a neuroprotective role in transient ischemia. Blocking the function of RANKL with an FDA-approved antibody (denosumab) has had striking success in reducing osteoporosis-related bone fragility that affects millions of women and has very few adverse side effects. With emerging evidence for the role of RANKL-RANK in progesterone-driven mammary tumors, denosumab might also hold great promise in slowing their progression and reducing the incidence of breast cancer. With the discovery of completely new activities of RANKL-RANK signaling on the horizon, especially in the context of the CNS, it is of great interest how the therapeutic inhibition of RANKL-RANK signaling could affect these novel functions, especially considering the neuroprotective role RANKL-RANK seems to play in the CNS under ischemic

conditions. It is not unreasonable, therefore, to assume that further detailed studies of this multifunctional triad will lead to new yet undiscovered functions and possible novel therapeutic targets for diseases previously not associated with RANKL-RANK signaling.

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