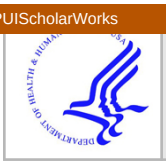

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RAGE signaling in Skeletal Biology

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Abstract

Purpose of review-—The receptor for advanced glycation end products (RAGE) and several of its ligands have been implicated in the onset and progression of pathologies associated with aging, chronic inflammation, and cellular stress. In particular, the role of RAGE and its ligands in bone tissue during both physiological and pathological conditions has been investigated. However, the extent to which RAGE signaling regulates bone homeostasis and disease onset remains unclear. Further, RAGE effects in the different bone cells and whether these effects are cell-type specific is unknown. The objective of the current review is to describe the literature over RAGE signaling in skeletal biology as well as discuss the clinical potential of RAGE as a diagnostic and/or therapeutic target in bone disease.

Recent findings-—The role of RAGE and its ligands during skeletal homeostasis, tissue repair, and disease onset/progression is beginning to be uncovered. For example, detrimental effects of the RAGE ligands, advanced glycation end products (AGEs), have been identified for osteoblast viability/activity, while others have observed that low level AGE exposure stimulates osteoblast autophagy, which subsequently promotes viability and function. Similar findings have been reported with HMGB1, another RAGE ligand, in which high levels of the ligand are associated with osteoblast/osteocyte apoptosis, whereas low level/short-term administration stimulates osteoblast differentiation/bone formation and promotes fracture healing. Additionally, elevated levels of several RAGE ligands (AGEs, HMGB1, S100 proteins) induce osteoblast/osteocyte apoptosis and stimulate cytokine production, which is associated with increased osteoclast differentiation/activity. Conversely, direct RAGE ligand exposure in osteoclasts may have inhibitory effects. These observations support a conclusion that elevated bone resorption observed in conditions of high circulating ligands and RAGE expression are due to actions on osteoblasts/osteocytes rather than direct actions on osteoclasts, although additional work is required to substantiate the observations.

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Conflict of Interest
Lillian Plotkin, Alyson Essex and Hannah Davis declare no conflict of interest.

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Summary-—Recent studies have demonstrated that RAGE and its ligands play an important physiological role in the regulation of skeletal development, homeostasis, and repair/regeneration. Conversely, elevated levels of RAGE and its ligands are clearly related with various diseases associated with increased bone loss and fragility. However, despite the recent advancements in the field, many questions regarding RAGE and its ligands in skeletal biology remain unanswered.

Keywords

RAGE; bone; osteoblast; osteoclast; osteocyte; osteoporosis

Introduction

The receptor for advanced glycation end products (RAGE) is a multi-ligand receptor belonging to the immunoglobulin receptor superfamily [1, 2]. RAGE binds numerous endogenous and exogenous ligands and plays a critical role in regulating the innate immune response [3]. Additionally, recent evidence suggests that RAGE signaling is involved in inflammation resolution, tissue homeostasis, and repair/regeneration following injury [1]. Despite this evidence, most studies have focused on the pathological role of RAGE signaling. RAGE is upregulated during the onset and progression of a variety of aging- and inflammation-associated diseases including diabetes, cancer, cardiovascular disease, neurodegeneration, and bone-related pathologies [4–6]. Based on these findings, the potential therapeutic effects of targeting RAGE and its ligands to treat/prevent inflammation-associated pathologies in humans is being investigated. This is supported by the fact that global RAGE knockout (KO) mice are viable and do not exhibit profound phenotypic abnormalities [7]. Numerous small molecule RAGE inhibitors have been developed over the last decade, and their research and clinical utility has been evaluated [3, 8]. Further, the diagnostic potential of RAGE and its ligands as biomarkers for the progression and severity of various pathologies has also been demonstrated [2, 9–12].

In the skeleton, RAGE plays a role in regulating bone metabolism under physiological conditions and may contribute to various bone-related diseases [4]. However, the specific cell type that mediates RAGE signaling, and the downstream effects of RAGE activation on bone homeostasis and pathology, remain unclear. Further, the therapeutic potential of targeting RAGE and its ligands and their diagnostic potential as biomarkers of bone-related pathologies has not been elucidated. In this review, we cover the current knowledge on this topic and examine the bone cell type-specific roles of RAGE signaling during bone metabolism and turnover.

Biology of RAGE and its Ligands

Receptor structure and isoforms

RAGE is ~50kDa protein consisting of an extracellular N-terminal V-type (variable) and two C-type (constant) domains, a single transmembrane domain, and a C-terminal cytoplasmic tail [1, 3]. The extracellular V-type domain is the receptor ligand binding portion and the cytoplasmic tail is required for signal transduction, however, adaptor protein association is required for signal transduction [1, 13].

Full-length (fl)-RAGE mRNA undergoes extensive alternative splicing that generates more than 20 isoforms, including membrane and soluble forms [14–16]. N-truncated RAGE is composed of the transmembrane and cytoplasmic domains, but lacks the V-type domain [14], whereas dominant negative RAGE contains the extracellular and transmembrane domains, but lacks the cytoplasmic domain, and is therefore able to bind ligands, but is unable to transduce signaling [16].

Further, two extracellular secreted forms of RAGE, endogenous secretory (es) and soluble (s) RAGE, have been identified [17]. esRAGE is generated by alternative splicing of RAGE mRNA, whereas sRAGE is released following proteolytic cleavage of fl-RAGE by ADAM10 and MMP9 [18]. Although the sRAGE isoforms lack the transmembrane and cytoplasmic domains, they retain their ligand binding ability, acting as decoy receptors preventing ligand binding to RAGE or other receptors [19]. Consistent with this notion, sRAGE treatment downregulates RAGE signaling and subsequently decreases inflammation [20, 21]. However, sRAGE administration can also stimulate signal transduction and induce an inflammatory response [22, 23].

RAGE signaling

RAGE is expressed in various cells types during tissue development, homeostasis, and regeneration/repair, suggesting a critical role of RAGE in these processes [1]. Upon ligand binding, RAGE activation stimulates downstream signaling pathways (NF-κB, AP-1, CREB, STAT3, NFAT) that induce cytokines/chemokines transcription, control cellular processes, and influence cell viability by regulating autophagy and apoptosis [1]. Cell type-related differences in adaptor protein signaling, as well as cell/tissue-specific gene transcription, are potential elements that contribute to cell type-specific effects of RAGE activation [24]. Additionally, factors such as metabolic status, reactive oxygen species (ROS) levels, and intra- and extracellular redox conditions, can also alter RAGE ligand properties and modify downstream signaling [1, 25, 26]. RAGE-ligand signaling and its downstream effects on skeletal biology are covered in more detail later in the review.

Aside from binding numerous ligands, RAGE activation alters numerous downstream signaling pathways in bone cells. In particular, RAGE is critical for αvβ3 integrin-mediated adhesion in osteoclasts [27]. Further, RAGE-deficient osteoclasts are not able to respond to vitronectin, a αvβ3-integrin ligand, and exhibit impaired PYK2 and ERK1/2 phosphorylation. Additionally, RAGE-deficient osteoclasts have reduced integrin expression, which is needed for proper osteoclast maturation and adhesion; they also have decreased c-Fos and NFATc-1 expression in response to M-CSF and RANKL signaling. Dia-1, a member of the formin family of proteins, also binds RAGE via an FH1 domain on the cytoplasmic tail of the receptor. This allows for signal transduction to elicit cellular migration using the GTP-ase activity of Rac-1 and Cdc42, known osteoclastogenic GTP-ases [13, 28]. In osteoblasts, AGE-RAGE activation increased Raf/MEK/ERK signaling, decreasing cell viability and activating beclin-1 and LC3B-mediated autophagy [29]. Further studies are needed to elucidate the multiple signaling pathways that might be activated by RAGE-ligand interaction, and the particular signaling cascade activated by different ligands in bone cells.

RAGE Ligands

RAGE is a pattern recognition receptor (PRR) that binds numerous endogenous and exogenous ligands [1, 30]. Aside from RAGE, other PRRs include toll-like receptors (TLRs), mannose receptors (MR), and NOD-like receptors (NLR), which bind overlapping ligands [31]. RAGE binds endogenous damage-associated proteins (DAMPs), including advanced glycation end products (AGEs), high mobility group box 1 (HMGB1), S100 calgranulin proteins, amyloid β peptide (Aβ), heat shock proteins (HSP), and macrophage 1 antigen (Mac-1/CD11b), that are released extracellularly in response to cell stress and death [4, 19]. Elevated extracellular RAGE ligand levels stimulate RAGE expression, mainly through NF-κB activation [32]. RAGE expression is upregulated in multiple cell types/tissues during the onset/progression of numerous pathological conditions [33]. In particular, RAGE expression and extracellular ligand levels play an important role in regulating bone metabolism and elevated RAGE-ligand signaling is associated with various pathologies characterized by reduced bone mass/strength [4]. Below we further detail the roles of the key RAGE-ligands during bone metabolism and pathology.

AGEs.—AGEs occur from aldose sugar-mediated non-enzymatic chemical modifications of proteins and lipids [30]. AGE formation and tissue accumulation increases during aging and conditions of hyperglycemia such as diabetes. Tissues containing long-lived proteins, such as collagen, and with low turnover are particularly vulnerable to such non-enzymatic modifications [34]. In bone, AGE accumulation alters the material properties and decreases toughness [35, 36]. Additionally, circulating AGEs can bind RAGE, as well as other receptors and stimulate signal transduction in various tissues/cell types [30]. AGE-RAGE signaling has been implicated in numerous aging-related pathologies [34]. In particular, it has been proposed that prolonged AGE signaling plays a role in the onset/progression of diabetic osteopenia and may also contribute to aging-related bone fragility [36].

HMGB1 (amphoterin).—HMGB1, first described as a chromatin-bound protein, is released by necrotic cells as a DAMP, and subsequently promotes monocyte recruitment and pro-inflammatory cytokine release [37]. HMGB1 signals through RAGE, and can also act through TLR4 and 2, producing a similar pro-inflammatory response, activating NF-κB through effectors such as TRAF6, IKKα, and IKKβ [38]. HMGB1/RAGE signaling promotes immune cell differentiation and proliferation [39], as well as cellular migration, probably in part due to the secretion of pro-inflammatory cytokines through NF-κB signaling [40]. HMGB1 is also extracellularly released by osteoclasts, osteoblasts, and osteocytes, which all express RAGE [41]. In primary osteoblasts and MC3T3-E1 osteoblastic-like cells, parathyroid hormone (PTH) treatment attenuates HMGB1 release, whereas in rat UMR 106–01 osteosarcoma cells PTH increases its release. Interestingly, both the increased and decreased HMGB1 release from these different cell lines is mediated by adenylyl cyclase, highlighting the diversity and context-specific effects of PTH-signaling. In osteoclasts, epidermal growth factor-mediated HMGB1 release induces RANK expression via CD68 in patients with autoimmune disease, suggesting that HMGB1 promotes osteoclast differentiation in human diseases [42].

S100 calgranulin proteins.—The S100 proteins, are a small (10–12kD), highly-conserved family of proteins characterized by a unique Ca2+ binding motif [43]. S100 proteins localize to specific cellular compartments and translocate upon Ca2+ signaling. S100 proteins signal through RAGE and/or TLR4, requiring Ca2+ to do so, and are released by leukocytes in high-inflammation disease states [44]. Different family members activate or inhibit the receptors in a tissue- and context-specific manner. Interestingly, S100 protein signaling via RAGE promotes the secretion of cytokines in endothelial cells and leukocytes through NF-kB signaling, resulting in a pro-inflammatory environment that contributes to disease pathology, for example, in chronic kidney disease [45]. In skeletal muscle, S100B promotes proliferation in low-density myoblast cultures, enhancing myogenic differentiation via p38/MAPK, [46] whereas in aging, S100B is increased and downregulates myogenic differentiation in skeletal muscle [46]. In bone, S100 proteins regulate skeletal metabolism through direct and indirect actions on bone cells, however more work is needed to understand the cell-type specific roles of S100 proteins [47, 48].

Amyloid Beta Fibrils (Aβ).—Amyloid precursor protein (APP), most famously studied in neurodegenerative diseases, aggregates into plaques as amyloid-β (Aβ) via enzymatic cleavage [49]. Aβ signals through RAGE, potentially due to its structural similarities to a glycoprotein [50]. In the brain, Aβ binding to RAGE signals through p38/MAPK and receptor-mediated protein uptake [51]. Aβ is also expressed in bone and influences bone cell activity. For example, γ secretase-dependent cleavage of APP alters osteoblast activity; and Aβ promotes RANKL-induced osteoclast differentiation via NF-κB, ERK, and calcium oscillation signaling [52]. Additionally, skeletal analysis in APPswe/Aβ transgenic mice found that Aβ regulates osteoclastogenesis and RAGE expression in an age-dependent manner [53]. Aβ peptide expression is also elevated in osteoporotic bone tissue, consistent with the pro-inflammatory/pathological effects of Aβ during aging and disease onset/progression [54, 55]. Further, the antioxidant curculigoside attenuates memory and bone loss in APP/PS1 transgenic mice, highlighting a potential involvement of Aβ signaling in bone loss [56].

Physiological RAGE Signaling in Bone

Bone cells [mesenchymal stem cells (MSCs), osteoclasts, osteoblasts, and osteocytes] express RAGE and its expression is differentially regulated during cell proliferation, differentiation, and survival [4]. Further, studies in global RAGE KO mice have demonstrated the importance of RAGE in the regulation of bone accrual and turnover [57, 58]. However, while it is clear that RAGE plays a critical role in the skeleton, the mechanisms underlying these effects and the cell-type specific roles of RAGE and its ligands in bone remain unclear.

Young (1–3-month-old) RAGE KO mice exhibit high bone mineral density (BMD), bone volume, and biomechanical strength compared to wildtype mice [57]. Consistently, whole body/vertebral BMD and vertebral cancellous bone volume are also higher in 4-month-old RAGE KO compared to wildtype mice [58]. However, femoral bone mass accrual was attenuated and femoral cancellous bone volume was reduced in the absence of RAGE. Further, RAGE KO mice maintain bone mass and exhibit reduced bone resorption following

ovariectomy (OVX) [59], but RAGE deficiency impaired PTH-induced increases in femur cancellous bone volume, whereas it did not prevent the effects of PTH on vertebral cancellous bone.

Consistent with the high bone mass of RAGE KO mice, *in vivo* and *in vitro* studies showed that osteoclast differentiation and activity are decreased in RAGE KO compared to wildtype mice [4, 57, 60]. Further, RAGE expression is increased during osteoclast differentiation and RANKL stimulates RAGE expression in osteoclasts in a time- and dose-dependent manner [4, 57]. Additionally, *in vitro* studies demonstrated that RANKL-mediated osteoclast differentiation/activity is attenuated in bone marrow cells isolated from RAGE KO mice and RAGE deficiency leads to morphological defects, which are associated with reduced osteoclast differentiation and bone resorption [60]. These osteoclast defects are due to defective αvβ3-dependent signaling and attenuated actin-based cytoskeletal organization in RAGE KO bone marrow-derived macrophages (BMMs) and pre-osteoclasts. Further, *in vitro* mechanistic studies demonstrated that RAGE is required for the signaling events that stimulate osteoclast differentiation and function, upon engagement of M-CSF and αvβ3 integrin-signaling. However, whether RAGE controls integrin signaling at the transcriptional, cell surface, or intracellular level remains unknown [57, 60]. Overall, these findings highlight the important role that RAGE signaling plays in regulating osteoclast development and activity.

RAGE signaling also appears to play an important role in osteoblasts and osteocytes, although this topic has been less studied. Consistent with the decreased femoral cancellous bone accrual and altered architecture detected in the 4-month-old RAGE KO mice, the expression of several osteoblast-associated genes, ALP, Cola1, Runx2, and Osterix was decreased in femurs from these mice [58]. Further, global RAGE deficiency suppresses PPARα and its co-factor PGC1α, which leads to a pro-inflammatory phenotype in bones and osteoblasts from RAGE KO compared to control mice [61]. It should be noted that the meaning of the “pro-inflammatory phenotype” was not clearly described in the Biswas study, making it hard to draw conclusions about the osteoblastic effects of RAGE deficiency. In addition to these findings, preliminary work by our group found that *in vitro* mineralization is decreased in bone marrow cells from global RAGE KO compared to age-matched wildtype control mice. While these findings point to an essential role of RAGE signaling in osteoblast metabolism and function, additional studies are needed to clearly elucidate RAGE roles in osteoblasts and the mechanisms mediating these effects. Further, more work is needed to determine whether RAGE signaling also regulates osteocyte viability/function.

Taken together, this evidence suggests that RAGE signaling plays an important role in regulating both osteoclast and osteoblast differentiation/activity. Further, RAGE signaling effects are both age-related and bone site-specific. Overall, while our understanding of the mechanisms that regulate the downstream effect of RAGE signaling in bone under both physiological and pathological conditions has advanced in recent years, many questions still remain unanswered.

RAGE Ligand Regulation of Bone Metabolism

Low dose/intermittent exposure

Consistent with the inhibitory effects of genetic RAGE deficiency in osteoblasts, several studies have identified beneficial effects associated with RAGE ligand stimulation and signaling in MSCs and osteoblastic cells (Figure 1A). In particular, HMGB1 signaling in MSCs/osteoblasts promotes tooth socket bone healing in mice following a tooth extraction [62]. Further, HMGB1 stimulates cytokine release and promotes osteogenic MSC differentiation [63]. Consistent with the beneficial effects of HMGB1 on bone repair, treatment of bone fracture sites with HMGB1 and MSC-containing gelatin sponges enhances fracture repair [64]. Additionally, HMGB1 drives osteoblast migration and promotes fracture site vascularization, which in turn stimulates endochondral bone formation [39, 65]. Hypoxic-conditions also induce HMGB1 expression/release, which subsequently promotes osteoblast proliferation [66]. Moreover, recent studies found that low levels of AGEs have beneficial effects on osteoblasts. One study demonstrated that low levels of AGE-RAGE signaling stimulates autophagy and enhances osteoblast viability [29]. These findings highlight the possibility that under physiological conditions short-term or low level RAGE ligand exposure may have protective effects in bone cells. This notion is consistent with the idea that chronic rather than acute RAGE signaling induced by inflammation/cell stress leads to disease onset/progression.

High dose/chronic exposure

Despite the beneficial effects of low-dose/short-term RAGE ligand exposure, high-dose exposure negatively affects bone metabolism (Figure 1A). In particular, AGE treatment in MSCs prevents proliferation/differentiation into osteoblasts and inhibits mineralization [67]. Further, AGEs increase ROS generation, stimulate RAGE expression, and induce MSC apoptosis [68, 69]. Similar effects of AGEs have been observed in osteoblasts and osteocytes. In hFOB1.19 osteoblastic cells, AGEs stimulate mitochondrial dysfunction and apoptosis [70–72]. Consistently, AGE exposure inhibits osteoblast proliferation/differentiation, decreases osteoblastic gene expression and suppresses mineralization and lysyl oxidase enzyme activity [73–76]. AGEs also stimulate apoptosis in MLO-Y4 osteocytic cells [77] and promote pro-inflammatory cytokine release (IL-6, TNFa, RANKL, VEGFA) from osteoblasts and osteocytes [78–80]. Additionally, HMGB1 and S100A9 have similar effects on cell viability and cytokine production/release in osteoblasts and osteocytes [47, 48, 81].

In particular, work by our group demonstrated that apoptotic MLO-Y4 osteocytes release elevated levels of RANKL and HMGB1 and prevention of apoptosis or blockade of HMGB1 actions attenuated RANKL expression/release [81]. Further, we found that conditioned media (CM) from apoptotic MLO-Y4 osteocytes induced osteoclast differentiation; and inhibition of apoptosis or blockade of HMGB1 actions in osteocytes attenuated these effects [81]. Further, inhibition of HMGB1 autocrine actions in osteocytes using neutralizing antibodies, followed by immune-precipitation (IP) to remove the antibody-HMGB1 complexes from the CM blocked the increases in osteoclastogenesis induced by CM from apoptotic Cx43-deficient MLO-Y4 cells. On the other hand, IP removal of HMGB1 after

collecting the osteocytic CM did not prevent osteoclast differentiation. These findings suggest that HMGB1 autocrine actions on osteocytes, rather than direct actions on osteoclasts mediate the pro-osteoclastogenic effects of HMGB1 (Figure 1B). Consistent with this notion, S100A9 treatment in osteoblasts stimulated RAGE expression and promoted cytokine release; and S100A9-treated osteoblast CM increased osteoclast differentiation/activity, whereas directly in osteoclasts S100A9 inhibited osteoclastogenesis [47].

Similar effects have also been found with other RAGE ligands. For example, while high levels of AGEs during chronic inflammation induces apoptosis and increases inflammatory cytokine release in osteoblasts/osteocytes, the direct effects of AGEs in osteoclasts is not as well understood. While some studies have suggested that AGEs induce osteoclast differentiation/activity, others have found that AGEs inhibit osteoclast differentiation/activity. In pre-osteoclasts, AGE treatment inhibits fusion/differentiation and decreases resorption, whereas in mature osteoclasts treatment slightly increases differentiation/activity [82]. On the other hand, AGEs increase osteoblasts/osteocytes cytokine release, which may subsequently increase osteoclastogenesis. Consistently, high levels of AGE in bone are associated with increased osteoclast activity in humans, despite a lack of effect of AGEs on osteoclast activity *in vitro* [83].

Overall, these findings suggest that, at least at high doses/chronic exposure, RAGE ligands (AGEs, HMGB1, S100 proteins) may directly induce apoptosis and inhibit bone cell differentiation/activity (Figure 1). Additionally, these findings suggest that RAGE-ligand signaling-induced apoptosis in osteoblast/osteocytes stimulates cytokine release, which may subsequently induce osteoclast differentiation/activation.

RAGE and its Ligands in Bone Disease

In addition to their physiological roles, RAGE and its ligands are involved in various pathologies characterized by reduced bone mass and increased fragility/fracture. RAGE expression and its ligands are upregulated in numerous pathologies characterized by systemic inflammation, which are also associated with bone loss [3]. Consistently, as discussed above, elevated RAGE expression and increased circulating levels of RAGE ligands alter bone cell differentiation, activity, and viability pointing to the potential involvement of RAGE signaling in the onset of bone loss in these pathologies [4].

In particular, RAGE and its ligands have been linked to several diabetes-associated conditions and elevated levels of circulating AGEs and increased RAGE signaling are associated with osteoporosis in diabetes [36]. AGEs negatively affect bone metabolism in diabetes; and decrease bone healing in a mouse model of type 1 diabetes (T1D) [84]. Consistently, bone marrow MSC maintenance is impaired in mouse models of both T1D and T2D; and RAGE KO mice are resistant to the streptozotocin T1D-induced decreases in MSCs [69]. These pieces of evidence highlight the potential involvement of RAGE expression/signaling during the onset and progression of diabetes-related bone loss.

RAGE as a Biomarker for Bone Disease/Osteoporosis

Due the presence of circulating soluble RAGE isoforms in serum/plasma, the diagnostic potential of sRAGE as a biomarker has been evaluated in various diseases [12]. However, to date the preventive and diagnostic potential of sRAGE in bone disease has been quite contradictory. Thus, no association between RAGE polymorphisms and bone fractures were detected in T2D patients, and no correlation between circulating sRAGE levels and osteoporosis was found [85].

However, findings from other studies have suggested that low levels of sRAGE may be indicative of bone disease and be associated with high bone resorption, due to the absence of the inhibitory decoy effects of sRAGE. Consistent with this possibility, low serum esRAGE levels is a risk factor for vertebral fractures [86]. Additionally, low serum sRAGE and S100A12 levels in juvenile idiopathic arthritis correlated with increased disease activity [87].

Several other recent studies have suggested that sRAGE levels may actually be elevated in osteoporotic patients. sRAGE levels were elevated in rheumatoid patients with high levels of bone and cartilage turnover prior to hormone replacement therapy (HRT) [88]. Following HRT, patients exhibited decreased sRAGE levels that correlated with increases in BMD. Additionally, elevated levels of circulating AGE and esRAGE were associated with increased bone turnover and hip fracture incidence in elderly men [89]. Further, circulating sRAGE levels significantly correlate with osteopenia and osteoporosis [90]. And, higher sRAGE levels positively associated with elevated FGF23 levels, a protein known to be elevated in osteoporotic patients. Interestingly, sRAGE levels also negatively correlated to BMI and leptin, suggesting sRAGE could be a biomarker indicative of both bone fragility and lipid metabolism. The authors of this study also speculated that elevated sRAGE could be a direct effect of increased MMP9 activity, suggesting that sRAGE may be an indicator of increased osteoclast activity rather than an inhibitor of bone resorption due to its decoy activity. Based on this notion, sRAGE may indicate high levels of bone turnover and may be higher when circulating DAMP levels are elevated. Interestingly, recent studies by our group found that esRAGE and TLR4, but not RAGE mRNA expression is increased in osteocyte enriched long bones of aged (21-month-old) compared to young (4-month-old) mice, suggesting that elevated membrane bound RAGE expression, at least in osteocytes, may not be responsible for promoting age-related bone loss and circulating sRAGE levels may actually be elevated in aging-induced osteoporosis.

Therapeutic Potential of Targeting RAGE and its Ligands

Based on the idea that chronic elevated RAGE signaling contributes to the onset/progression of bone disease in aging and conditions of increased inflammation, several studies have examined the effects of targeting/blocking RAGE and its ligands in bone. Due to its decoy inhibitory properties, the therapeutic potential of sRAGE has been examined by several groups [91]. Additionally, blockade of RAGE signaling by sRAGE administration has beneficial effects in mouse models of Alzheimer’s disease [92]. In MSCs, sRAGE transfection prevents HMGB1-induced inflammation [93]. Further, RAGE inhibition by sRAGE treatment also had protective effects on bone metabolism and diminished alveolar

bone loss in a mouse model of diabetic periodontal disease [91]. Despite the anti-inflammatory effects following sRAGE administration, other studies have seen cell/tissue-type specific pro-inflammatory effects with sRAGE [18, 22, 23].

In addition to sRAGE, numerous small-molecule inhibitors that target RAGE and its ligands have developed and evaluated over the last decade [8]. For example, the small molecule TTP488 (Azeliragon) inhibits RAGE ability to bind ligands, including AGEs, HMGB1, S100 proteins, and Aβ [94]. The effects of this compound in Alzheimer's disease have been evaluated in both pre-clinical mouse models and clinical trials [95, 96]. In addition, screening a library of small molecule RAGE inhibitors, FPS-ZM1, a compound that inhibits RAGE-Aβ interactions was identified [97]. Pre-clinical studies with FPS-ZM1 showed beneficial effects in many disease states, including neuro-inflammation and cancer. Additionally, FPS-ZM1 has protective effects in bone by preventing RAGE-dependent mitochondrial dysfunction and apoptosis in osteoblasts and osteocytes [72, 78]. These findings highlight the therapeutic potential of small-molecule RAGE inhibitors to treat and/or prevent bone disease.

Conclusions

RAGE and its ligands play an important physiological role in the skeleton, and elevated levels of RAGE and its ligands are clearly related to various bone-related diseases. Despite the recent advances in this field, many remain questions unanswered: 1) What are the specific roles that RAGE and its ligands play in the various bone cell types under physiological and pathological conditions? 2) What is the therapeutic potential of RAGE and its ligands as pharmacological targets to prevent bone loss/fragility induced with aging and inflammatory diseases? 3) Could RAGE and its ligands provide diagnostic utility serving as biomarkers of bone disease progression and severity? Thus, while our understanding of RAGE signaling in the skeleton has improved, several key questions remain to be answered and warrant further investigation.

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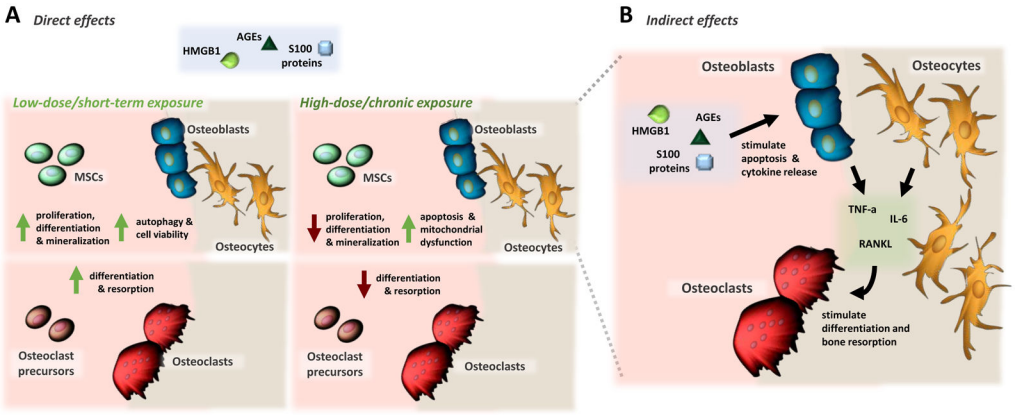

Figure 1: RAGE ligands signaling in bone cells: direct and indirect effects.

Schematic illustration of the dose and bone cell-type specific effects of RAGE-ligand signaling in the skeleton. (A) Direct bone-cell type specific effects of low-dose/short-term and high-dose/chronic RAGE ligand exposure. (B) Indirect and paracrine-signaling bone microenvironment changes induced by RAGE ligand-signaling.