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Targeting RAGE Signaling in Inflammatory Disease

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Abstract

The receptor for advanced glycation end-products (RAGE) is a multiligand pattern recognition receptor implicated in diverse chronic inflammatory states. RAGE binds and mediates the cellular response to a range of damage-associated molecular pattern molecules (DAMPs) including AGEs, HMGB1, S100s, and DNA. RAGE can also act as an innate immune sensor of microbial pathogen-associated molecular pattern molecules (PAMPs) including bacterial endotoxin, respiratory viruses, and microbial DNA. RAGE is expressed at low levels under normal physiology, but it is highly upregulated under chronic inflammation because of the accumulation of various RAGE ligands. Blocking RAGE signaling in cell and animal models has revealed that targeting RAGE impairs inflammation and progression of diabetic vascular complications, cardiovascular disease (CVD), and cancer progression and metastasis. The clinical relevance of RAGE in inflammatory disease is being demonstrated in emerging clinical trials of novel small-molecule RAGE inhibitors.

INTRODUCTION

RAGE: receptor for advanced glycation end-products

AGE: advanced glycation end-product

PRR: pattern recognition receptor

The receptor for advanced glycation end-products (RAGE) is a transmembrane protein belonging to the immunoglobulin (Ig) superfamily of receptors (1). Initially identified by its ability to bind and mediate the effects of advanced glycation end-products (AGEs) that underlie diabetic complications, RAGE can bind a diverse class of non-AGE ligands. Due to its ability to recognize a range of structurally unrelated endogenous and exogenous ligands, RAGE was termed a pattern recognition receptor (PRR) (2). RAGE has emerged as a key regulator of the innate immune response. Under conditions of chronic and sustained inflammation, RAGE signaling is upregulated, leading to the development and progression of pathological states including diabetic vascular complications, cardiovascular disease (CVD), cancer, Alzheimer's disease (AD), and a range of host- and pathogen-induced inflammatory diseases (3, 4). As RAGE knockout mice are viable and appear physiologically normal (5), therapeutic targeting of RAGE in humans may be safe and effective. Recently, a variety of small-molecule RAGE antagonists has become available both for research and for clinical use (6, 7). This review focuses on the pathobiology of RAGE in inflammatory disease, and the emergence of clinical and translational studies on RAGE.

THE BIOLOGY OF AGES AND RAGE

RAGE was first isolated from bovine lung in 1992 and named for its ability to act as a receptor for AGEs (1). AGE formation occurs during aging and at an accelerated rate under conditions of hyperglycemia and oxidative stress, as in diabetes (3). AGEs are a heterogenous group of molecules that are formed by the nonenzymatic reaction between reducing sugars and proteins, and also lipids and DNA (8). Also known as the Maillard reaction, nonenzymatic glycation results in the formation of early reversible products known as Schiff bases and Amadori products (8). The first glycation product to be characterized in humans was the Amadori product hemoglobin A1c (HbA1c), which is now used clinically as an indicator of long-term glycemic control in diabetic patients (9). Clinical studies including the Diabetes Control and Complications Trial and United Kingdom Prospective Diabetes Study have demonstrated that when HbA1c levels are maintained below 7.5%, the risk of developing retinopathy, nephropathy, neuropathy, and CVD is lowered (10, 11). Amadori products subsequently undergo a series of structural and oxidative reactions to form the highly stable and irreversibly bound AGEs (8).

AGEs are prevalent in the diabetic vasculature and contribute to microvascular and macrovascular complications by forming cross-links with structural proteins in the basement membrane and by engaging RAGE (1, 3). While these extracellular AGEs form at a slow rate in basement membrane collagens and lens crystallins (8), intracellular AGEs originating from glycolytic intermediates can form more rapidly (12). Intracellular-formed AGEs include N epsilon-(carboxymethyl)lysine (CML) and N epsilon-(carboxyethyl)lysine (CEL). CML is the most prevalent AGE in diabetics and is a proven RAGE ligand (13). RAGE was initially thought to be an AGE scavenger receptor and act as a clearance and degradation mechanism (1). However, it is now accepted that RAGE acts as a signaling receptor leading to changes in gene expression and cellular properties (3, 14).

RAGE is a type 1 transmembrane glycoprotein and a member of the Ig superfamily of receptors due to the presence of multiple Ig-like domains in its extracellular region (**Figure 1**). The extracellular region contains three Ig domains including a V-type domain, a C1-type domain, and a C2-type domain. Binding of ligands has been shown to predominantly occur in the V domain (14). The extracellular region is followed by a single transmembrane region and a short, highly charged cytoplasmic domain that is critical for signaling (3, 15). Protein homology studies reveal that RAGE is present only in mammals and has the highest homology with genes coding for Ig

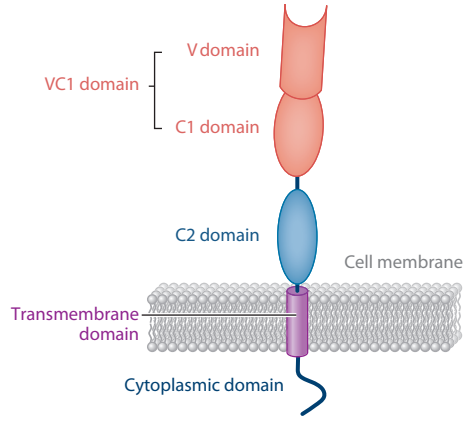

Figure 1

Schematic representation of RAGE (receptor for advanced glycation end-products) and its major protein domains. RAGE is composed of an extracellular region containing three immunoglobulin (Ig) domains, a single transmembrane domain, and an intracellular cytoplasmic domain. The Ig domains are a V-type, C1-type, and C2-type. Structural studies have revealed the V and C1 domains are joined together to form the VC1 domain (shown in light red) and connected to the C2 domain (shown in blue) by a short stretch of amino acids that act as a flexible linker.

cell adhesion molecules (IgCAMs), including ALCAM, BCAM, and MCAM (16). IgCAMs possess multiple functions, including acting as ligand-binding signaling receptors (17).

The RAGE gene is located on chromosome 6 in the major histocompatibility complex (MHC) class III region, which contains many genes critical to both the adaptive and innate immune systems (18). RAGE is composed of 11 exons and is highly conserved across different mammalian species at both the DNA and protein levels (16). The RAGE gene undergoes extensive alternative splicing, resulting in more than 20 different splice variants; however, the predominant RAGE protein isoform in vitro and in vivo is the canonical version (19, 20).

Most studies have focused on the role of RAGE in disease states. The hallmark of RAGE in disease states is its striking upregulation in chronic inflammation. RAGE is expressed at low levels in most organs except the lung; it is highly expressed in type I alveolar (AT-1) epithelial cells (21). In AT-1 cells, RAGE is found at the basolateral membrane, where it acts as an adhesion molecule to mediate the contact between AT-1 cells and the lung basal lamina, and to contribute to their flat and spread morphology, which is essential for gas exchange (21). These data suggest that in addition to mediating the cellular responses to AGEs, RAGE may bind other epitopes.

RAGE Is a Multiligand Receptor

Shortly after RAGE was characterized as an AGE receptor, it was shown to bind a number of endogenous non-AGE ligands (22–24). RAGE binds diverse classes of ligands based on charge and multimeric state and is classified as a PRR (2). PRRs are a critical component of the innate immune system, sensing exogenous pathogenic agents and endogenous "danger" signals and activating inflammatory responses (25). PRRs include toll-like receptors (TLRs), nucleotide-binding oligomerization-like receptors, and various other DNA sensors. PRRs recognize exogenous pathogen-associated molecular patterns (PAMPs) from microorganisms, as well as danger-associated molecular patterns (DAMPs) released by cells under conditions of stress, injury, or cellular death (25). Whereas other PRRs recognize specific classes of PAMPs

PAMP: pathogen-associated molecular pattern

DAMP: danger-associated molecular pattern

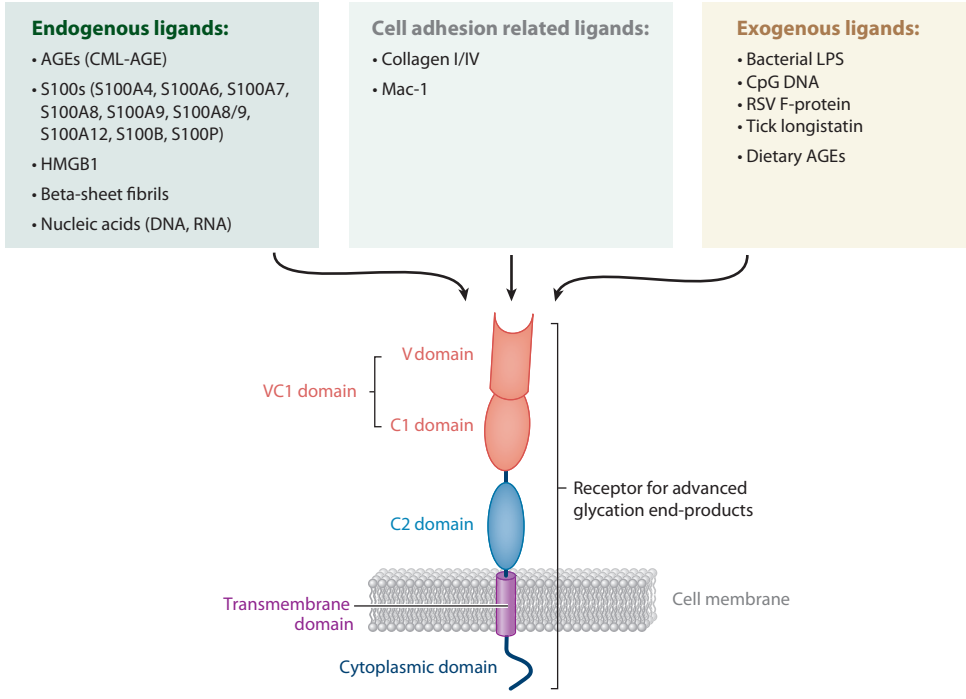

Figure 2

RAGE (receptor for advanced glycation end-products) is a multiligand pattern recognition receptor. RAGE binds a diverse class of ligands including those of an endogenous (damage-associated molecular pattern and adhesion-related proteins) and exogenous (pathogen-associated molecular pattern and dietary AGEs) nature. RAGE binding for the majority of ligands occurs in the V or VC1 extracellular domain of RAGE. Abbreviations: AGE, advanced glycation end-product; CML, N epsilon-(carboxymethyl)lysine; HMGB1, high mobility group 1; LPS, lipopolysaccharide; RSV, respiratory syncytial virus.

and DAMPs, RAGE appears to be able to bind and mediate cellular effects of a diverse group of endogenous and exogenous ligands (**Figure 2**).

Endogenous RAGE ligands.

HMGB1. HMGB1 (high mobility group 1, also known as amphoterin) was the first non-AGE ligand identified to bind to RAGE (22). Originally described as a DNA-binding protein involved in chromatin remodeling, HMGB1 is also a DAMP when released by cells (26). HMGB1 is actively secreted by various immune cells, especially monocytes and neutrophils, in response to stimulation by endotoxin [lipopolysaccharide (LPS)], CpG DNA, tumor necrosis factor (TNF)-α, and interleukin (IL)-1 (27). HMGB1 was first described as a proinflammatory cytokine and late mediator of delayed endotoxin lethality (28). It is passively released by dying cells, where it acts as a DAMP, stimulating further proinflammatory cascades and recruitment of immune cells (27). HMGB1 has been shown to activate proinflammatory effects through binding to RAGE on multiple cell types including monocytes, neutrophils, endothelial cells, smooth muscle cells, and cancer cells (22, 27). HMGB1 can also mediate proinflammatory effects through binding to other PRRs including TLR2 and TLR4 (27). Further studies are needed to dissect the overlapping cross-talk induced by HMGB1 between RAGE and TLRs.

S100/Calgranulins. The S100 family consists of 21 structurally related members that display diverse functions and expression patterns (29). S100s are small proteins that are characterized by two EF-hand motifs and have key roles inside the cell as calcium sensors, cell growth and differentiation modulators, and organizers of the actin cytoskeleton (29). S100s are released by multiple cell types in inflammatory states, where they act in a cytokine-like manner and as DAMPs. S100A12, also known as Calgranulin C, was the first S100 to be identified as a RAGE ligand (24). S100A12 (termed at the time "extracellular newly identified RAGE-binding protein" or EN-RAGE) was found to bind and activate RAGE signaling, leading to proinflammatory gene expression and cellular effects (24). RAGE has since been shown to bind and mediate effects of other S100s, including S100A1, S100A2, S100A4, S100A5, S100A6, S100A7, S100A8, S100A9, S100A11, S100A13, S100B, and S100P (24, 29–31). Even though they are similar in composition, not all S100s are ligands for RAGE (30). Protein structural studies of RAGE and S100s have suggested that S100 proteins capable of forming homo- or heterodimers leading to oligomerization may be required to bind to RAGE (30, 31). It is still not fully clear what the biological functions of all extracellular S100s are; however, the calgranulin subset of S100s is the best understood (30, 32). S100 calgranulins are mainly expressed and released by myeloid cell types and include S100A8 (Calgranulin A, also known as MRP8), S100A9 (Calgranulin B, also known as MRP14), and S100A12 (Calgranulin C) (32). S100A8 and S100A9 are predominantly present in the extracellular space as a heterodimer (S100A8/9), also known as calprotectin. S100A8/9 is predominantly expressed by neutrophils and makes up 30% or more of neutrophil cytoplasmic proteins (33). Calprotectin is highly upregulated in inflammatory bowel disease (IBD), and because of its extreme stability in fecal samples, it can be used as a biomarker of intestinal inflammation (34). As calprotectin levels are not elevated in irritable bowel syndrome (IBS), measuring fecal calprotectin has been proposed as a noninvasive alternative to endoscopy to differentiate IBS from IBD (34).

Other endogenous ligands. Other endogenous RAGE ligands have been identified, most of which are DAMPs that activate inflammatory responses. RAGE binds amyloid-β peptides (Aβ40 and Aβ42) that form aggregates in the brain and are the main component of amyloid plaques in AD (23). Thus, RAGE may function as a mediator of disease progression in AD. In endothelial cells of the blood–brain barrier (BBB), RAGE binds Aβ and facilitates its transport into the brain (35). In vitro and animal models indicate that, once in the brain, Aβ can induce neuroinflammation through RAGE (23, 35).

A major function of PRRs is to act as DNA sensors by recognizing host and foreign nucleic acids (DNA and RNA) (25). RAGE binds extracellular DNA and mediates its uptake into the cell, where RAGE interacts with the DNA sensor TLR9 in endosomal compartments (36). Unlike other DNA sensors, which are present in either the cytosol or endosomal compartment, RAGE appears to be a unique cell-surface DNA sensor (36). Interestingly, RAGE activation by DNA is stronger and more sustained when HMGB1/DNA complexes bind RAGE than when HMGB1 or DNA acts alone (37).

Exogenous RAGE ligands. The hallmark of PRRs is their ability to bind and activate the innate immune system in response to foreign molecules from bacteria, viruses, and parasites (25). RAGE also possesses the ability to recognize and respond to various PAMPs, including bacterial LPS, bacterial DNA, and viral and parasitic proteins (36, 38–40). LPS released by Gram-negative bacteria is the major cause of septic shock (38). RAGE can directly bind LPS and activate proinflammatory signaling independent of the major receptor for LPS, TLR4 (38). Although no studies have been performed on RAGE with bacterial or viral DNA, RAGE is able to bind and mediate effects of

the synthetic single-stranded DNA molecules [CpG oligodeoxynucleotides (CpG ODN)] that are used as artificial mimics of unmethylated microbial DNA (36). RAGE has been shown to bind and aid in the infection of AT-1 cells by respiratory syncytial virus (RSV), a major cause of respiratory tract infections in humans (39). RAGE binds the RSV fusion (F) protein, which allows RSV to infect airway epithelial cells (39).

Anisuzzaman et al. (40) demonstrated that longistatin, a protein secreted in the saliva of the *Haemaphysalis longicornis* tick, suppresses the host immune response through binding RAGE. Antagonism of RAGE by longistatin prevents endogenous DAMP ligands, including S100A8/9, from binding to RAGE. This suppresses RAGE inflammatory signaling in response to tissue injury induced by tick bites, and thereby helps the tick feed on the host's blood (40).

There is emerging evidence that exogenous dietary AGEs play a role in AGE-related pathology (41). AGEs are particularly abundant in foods that are processed and cooked. Indeed, the Maillard reaction and its products are known to be responsible for food colors and flavors. Therefore, heavily processed, burnt, and sugar-cured foods could be a major source of AGEs (41). Rodent studies show consumption of AGE-rich diets is associated with vascular inflammation and accelerated atherosclerosis (42, 43). However, it is not fully understood how dietary AGEs are absorbed or metabolized, or how they accumulate after ingestion. Long-term clinical studies are also needed to establish whether dietary AGEs impact subjects at risk of AGE-related disease states.

Protein structure studies reveal the multiligand function of RAGE. Understanding how RAGE binds and initiates signaling is critical in order to design effective therapeutics against the receptor. With such a repertoire of different ligands, knowledge of the protein structure of RAGE may not only provide vital information for rational drug design but also shed light on how RAGE acts as a PRR. Most protein structure studies of RAGE have focused on the extracellular or ligand-binding domain, in part due to the difficulty of crystallizing the transmembrane and intracellular highly charged signaling regions (14, 31, 44). Structural analyses have revealed that the extracellular region can be divided into two major subdomains: a structural unit formed between the V and C1 domains (VC1), connected to the C2 domain by a flexible linker of seven amino acids. The VC1 domain displays a net positive charge due to the high content of lysine and arginine residues in the V domain. These form a positive patch on the surface of VC1 (44). Indeed, the V domain of RAGE contains more arginine and lysine than any other Ig-V-containing protein (14, 44). In contrast, the membrane extracellular proximal region C2 domain is composed of acidic amino acids and displays a negative charge on its surface (14, 44).

The major common features of RAGE ligands are that they contain regions of high negative charge and can form multimeric forms. It is therefore no surprise that most RAGE ligands bind the positively charged VC1 domain (14). Contradictory reports exist as to whether S100A6 interacts with C2 or VC1 (45, 46).

It appears that multimeric RAGE is the predominant form of RAGE to mediate ligand binding, and this occurs predominantly by the RAGE VC1 domains. The RAGE VC1 domain facilitates ligand binding through a combination of the positively charged residues and a hydrophobic pocket created by the RAGE VC1 homodimer (14, 44) (**Figure 3**). RAGE dimerization occurs in a multimodal manner depending on amino acid residues in the V and transmembrane domains (47, 48). RAGE apparently preassembles to form constitutive multimers at the cell surface consisting of four or more RAGE molecules, prior to ligand presence and binding (4, 14). As monomeric RAGE displays relatively weak affinity for monomeric ligands, it has been proposed that the multimerization of RAGE is necessary to bind aggregates of ligands seen in inflammation (4, 14). Due to the strong electrostatic nature of RAGE ligand multimers, it has been proposed that these receptor–ligand complexes are tightly bound and lead to sustained activation of RAGE ligand

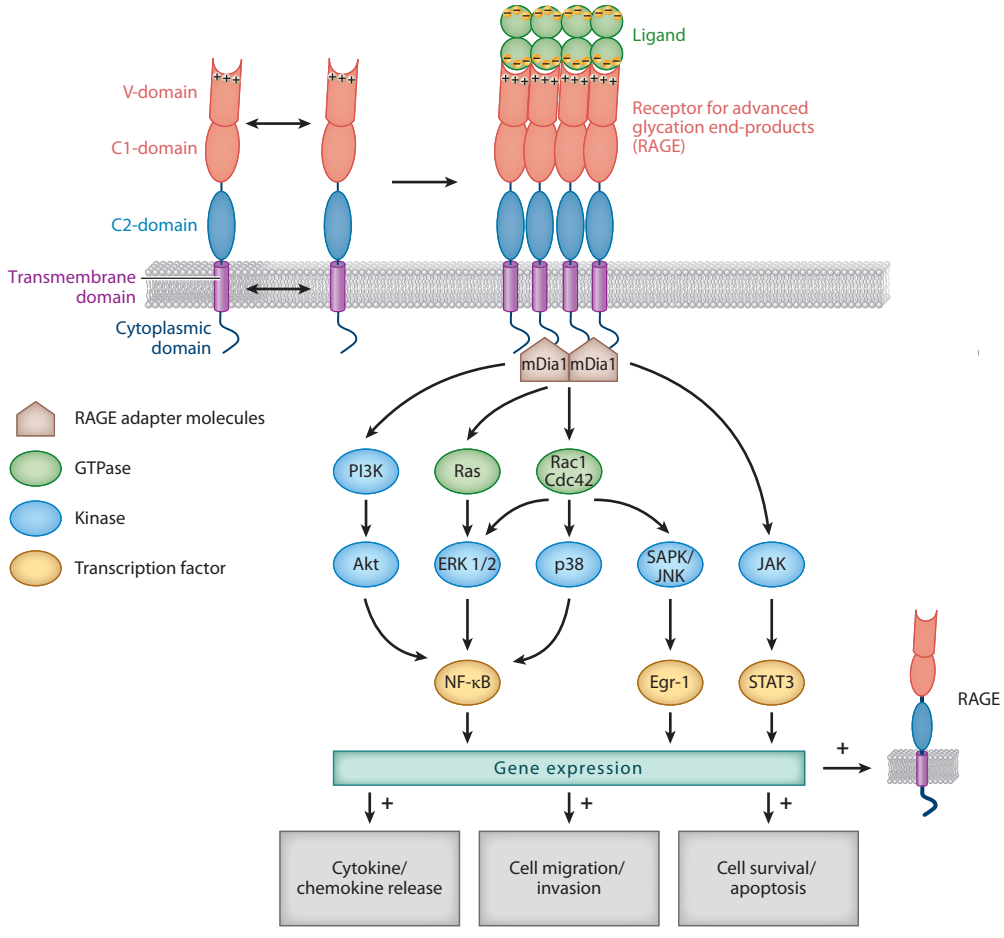

Figure 3

Activation of RAGE (receptor for advanced glycation end-products) signaling. Preassembly of RAGE into dimers and multimers is necessary for RAGE to activate downstream signaling pathways leading to changes in gene expression and cellular function. RAGE activates intracellular signaling by preassembling into dimers and multimers at the cell surface, prior to ligand binding. Multimeric RAGE binds ligands through electrostatic interactions between the positively charged (shown as +) VC1 domain of RAGE and the negatively charged (shown as –) property of ligands. Upon ligand binding, conformational changes in the intracellular domain of RAGE allow interaction with its intracellular partners including mDia1 (shown in brown). RAGE signaling occurs through Rho GTPases (shown in green) and kinases (shown in blue) that activate transcription factors (shown in yellow). RAGE activation of these signaling pathways leads to changes in gene expression and altered cellular function including migration, survival, inflammation, and upregulation of RAGE expression itself. Abbreviations: PI3K, phosphoinositide 3-kinase; ERK, extracellular signal–regulated kinase; SAPK/JNK, stress-activated protein kinases/Jun amino-terminal kinases; JAK, Janus kinase; NF-κB, nuclear factor kappa-light-chain enhancer of activated B cells; Egr-1, early growth response protein 1; STAT3, signal transducer and activator of transcription 3.

signaling (4, 14). These studies reveal that RAGE inhibition may work not only by preventing ligand binding but also by preventing the assembly of RAGE multimers at the cell surface.

RAGE Ligand Signaling

RAGE signaling pathways are governed by many factors, including the ligand and the cell type involved, the ligand concentration, multimeric forms of ligands, the presence of multiple RAGE

ligands, and the presence of other PRRs that can also bind these ligands (3, 4). RAGE activates various intracellular signaling pathways, including mitogen-activated protein (MAP) kinases (ERK 1/2, p38, SAPK/JNK), STAT3, Akt, and Rho GTPases (Rac1, Cdc42). These in turn activate downstream transcription factors, including NF-κB, EGR-1, and SP-1 (3, 4, 49). The pathways result in the expression of a range of proinflammatory genes, including VCAM-1, IL-6, TNF-α, and other immune modulators (3, 4, 49) (**Figure 3**).

Elegant molecular studies have established RAGE's involvement in cell signaling by expressing a genetically engineered isoform of RAGE lacking the cytoplasmic domain. This so-called dominant-negative or DN-RAGE impairs downstream signal transduction and also blocks cellular induced effects of RAGE ligands in vitro and in vivo (15, 24, 50, 51). We recently showed that an endogenous isoform of DN-RAGE occurs in normal biology due to alternative splicing of RAGE mRNA (52). This endogenous truncated isoform of RAGE (RAGEΔICD), which lacks most of the cytoplasmic domain, inhibits RAGE signaling and cellular function and may therefore act as a negative endogenous regulator of RAGE signaling (52).

The cytoplasmic domain of RAGE is a short 43-amino-acid region that shares no homology with other receptor cytoplasmic domains and does not harbor endogenous receptor tyrosine kinase activity (15). RAGE appears to activate signaling through the binding of adaptor proteins that include diaphanous-1/mDia1, ERK1/2, PKCζ, TIRAP, and DOCK7 (15, 53–55). Currently, the only direct binder to the RAGE cytoplasmic domain is mDia1. Identified by yeast-2-hybrid to bind to the RAGE cytoplasmic domain, mDia1 mediates downstream signaling through activation of MAP kinase activation and Rac-1/Cdc42 (15, 56). Crystal structure of RAGE/mDia1 revealed that RAGE amino acids R366 and Q367 interact with the FH1 domain of mDia1 (56). The RAGE cytoplasmic domain can be phosphorylated by PKCζ at S391, which regulates activation of downstream kinases (54). However, little is known of the functional consequences of this post-translational modification.

THE ROLE OF RAGE IN INFLAMMATORY DISEASE

RAGE and its ligands are a major inflammatory driving mechanism in the pathogenesis of diabetic complications, CVD, neurodegenerative conditions, cancer, and a range of other inflammatory states.

Diabetes and Cardiovascular Disease

Uncontrolled hyperglycemia in diabetes leads to the accumulation of AGEs, which drive vascular complications including nephropathy, retinopathy, neuropathy, and accelerated CVD. As RAGE was first identified as a receptor for AGEs, most preclinical and clinical studies on RAGE are focused on the diabetic state. One of the first studies tested the impact of RAGE inhibition on vascular permeability (57). Vascular leakage as a result of increased vessel permeability is a predictor of future vascular complications in diabetes. Two different approaches were used to block RAGE: blocking antibodies raised against the extracellular region of RAGE, and a recombinant form of RAGE comprising the extracellular region, termed soluble RAGE (sRAGE). In a rat model of type 1 diabetes, administration of anti-RAGE antibodies or sRAGE blocked the increased vascular permeability (57). These studies demonstrate the feasibility of RAGE inhibition as a means of avoiding diabetic complications.

In patients with diabetes, accelerated atherosclerosis leading to CVD is the major cause of mortality. To test the role of RAGE in this setting, atherosclerosis-prone apoE-null mice made diabetic with streptozotocin were treated with sRAGE (58). sRAGE treatment strikingly reduced the size and complexity of atherosclerotic lesions, as well as the accumulation of AGEs

and S100s in the vessel wall (58). These experiments have been replicated in type 2 diabetic models (59). Further, in both diabetic and nondiabetic apoE-null mice, RAGE gene knockout is protective against atherosclerosis development (59–61). RAGE drives atherosclerosis by participating in mechanisms that drive inflammation and plaque progression. RAGE mediates inflammatory cell recruitment to the atherosclerotic plaque through release of DAMPs at the plaque site (3). Further, through the binding of leukocytes to the endothelium via a Mac-1/RAGE interaction, RAGE is directly involved in leukocyte transmigration across the vessel wall (2). RAGE inhibition by sRAGE or RAGE gene ablation is protective in a range of CVD states including arterial restenosis, abdominal aortic aneurysm, cardiac injury after ischemia, and heart failure (50, 62).

The major microvascular complications of diabetes affect the kidneys, eyes, and peripheral nerves. In diabetic nephropathy models and in human clinical specimens, RAGE and its ligands are upregulated in podocyte cells of the glomerulus (63). Blocking RAGE activation with either sRAGE or gene knockout impaired renal injury and development of nephropathy (64). In murine models of retinopathy, RAGE blockade with sRAGE reduced the development of capillary lesions in the retina and impaired neuronal dysfunction (65). In neuropathy, RAGE inhibition or gene knockout attenuated electrophysiological and morphological changes in diabetic mice (66). Administration of sRAGE to mice restores impaired wound healing in diabetes (67), thus demonstrating a role for RAGE in multiple facets of diabetic complications where RAGE and its ligands accumulate.

Neurodegenerative Conditions

RAGE protein expression is elevated in human brain tissue in many inflammatory neurodegenerative states including AD, familial amyloid polyneuropathy, Parkinson's disease, inflammation following spinal cord injury, and diabetic neuropathy (23, 35, 68). Targeting RAGE may be an effective therapeutic approach in AD as RAGE drives neuroinflammation in response to Aβ accumulation in the brain. Either sRAGE or RAGE knockout that impairs RAGE signaling improves cerebral blood flow, cognitive decline, and neuroinflammation (68).

Cancer

RAGE has been implicated in the pathogenesis of a variety of cancer types including breast, glioma, bladder, melanoma, liver, pancreatic, prostate, colorectal, gastric, and lung (51, 69–72). Increased RAGE protein expression has been associated with increased tumor histological grade and poorer outcomes in a similar set of cancers (70, 73–76). RAGE alters properties associated with the malignant process, including increased cell migration and invasion, proliferation, and resistance to apoptosis (15, 51, 69, 71, 72). Blocking RAGE signaling in cancer cells reduces tumor growth both in vitro and in murine models, and therefore represents an attractive therapeutic target in cancer (15, 51, 70, 72). Importantly, RAGE inhibition and gene knockout have been shown to impair tumor metastasis (51, 69, 71), revealing a major new target for treating metastatic disease.

RAGE has been shown to affect tumor malignancy through other cells of the tumor microenvironment (77–79). In both chemically induced skin carcinogenesis and colitis-associated cancer models, RAGE gene knockout reduced tumor inflammatory cell recruitment and tumor progression (78, 79) by decreasing recruitment of myeloid precursor cells and macrophage polarization (77, 79). RAGE therefore is a highly attractive therapeutic target in cancer, as inhibiting RAGE affects not only the tumor cells but also numerous cell types of the tumor microenvironment that are critical for tumor progression and metastasis.

Other Inflammatory Disease States

RAGE signaling and RAGE ligands are upregulated in a range of other inflammatory conditions. Various groups have investigated the role of RAGE in rheumatoid arthritis, endotoxemia and septic shock, inflammatory bowel disease, and other pathological states (38, 80, 81).

RAGE AS A BIOMARKER FOR INFLAMMATORY DISEASE

RAGE and its ligands have been shown to be a potential biomarker in serum and tissue for disease progression and severity. These studies are beyond the scope of this review and are reviewed in depth elsewhere (3).

The major variants in the RAGE gene include a single-nucleotide polymorphism (SNP) encoding a protein coding change in the V domain (Gly82Ser) and two promoter variants (–374T/A and –429T/C) (3). Genome-wide association studies have linked the Gly82Ser variant with impaired airflow in people with chronic obstructive pulmonary disease (82, 83). The –374A allele, which has been shown to affect gene transcription, has been associated with lower incidence and lower risk of CVD (3). There are no known examples of genetic inactivation or, in cancer, the loss or gain of the chromosomal regions containing RAGE. In mice, deletion of the RAGE gene is not lethal, and knockout mice are developmentally normal (5). Although RAGE is highly expressed in the adult lung, RAGE knockout does not affect lung development or alter the life expectancy of mice (5). In fact, in aged-mice studies, RAGE knockout appears to extend the lifespan, as knockout mice reached the age of 24 months and older at a higher number than their wild-type littermates (84).

Soluble RAGE occurs endogenously and is formed by both alternative splicing and ectodomain shedding of cell-surface RAGE (85, 86). It is measurable in human serum and has been shown to be inversely correlated with CVD (87), diabetic complications, and a range of inflammatory states (3). A better understanding of the major isoforms and sources of sRAGE is still needed.

RAGE INHIBITORS

Unlike other PRRs, such as TLRs, which are highly conserved from insects to vertebrates and critical for regulation of immunity, RAGE is evolutionarily recent and present only in mammals (16). It therefore would appear RAGE evolved as an adhesion molecule and subsequently became a PRR immune sensor. The fact that RAGE gene knockout mice appear to be healthy and developmentally normal suggests RAGE inhibition to be a safe therapeutic approach.

Extensive studies in mice with various inhibitors of RAGE, predominantly excess levels of sRAGE decoys, have not shown any detrimental effects of RAGE inhibition. However, administration of sRAGE in humans may not be the ideal method to therapeutically target RAGE. As sRAGE is a large recombinant protein, production of therapeutic usable levels would be difficult and expensive. Additionally, because sRAGE is a ligand decoy, sRAGE administration would prevent ligand binding to receptors that share ligands with RAGE. As a consequence of these caveats, small-molecular inhibitors have been developed that target either the extracellular ligand-binding site or, more recently, the intracellular signaling domain of RAGE.

Extracellular Inhibitors of RAGE

TTP488 and derivatives. TTP488 is an orally bioavailable small-molecule inhibitor of RAGE. It is also known as PF-04494700 or azeliragon, and its chemical name is 3-[4-[2-butyl-1-[4-(4-chlorophenoxy)phenyl]imidazol-4-yl]phenoxy]-N,N-diethylpropan-1-amine (88). The majority of published information on TTP488 comprises human clinical studies (6, 88–90). Preclinical

in vitro and mouse model studies have been hampered by the lack of commercial availability of TTP488. Although most preclinical studies on RAGE have focused on diabetic complications, CVD, and cancer, the majority of clinical trial work with TTP488 has been in AD cohorts (6, 88, 89). TTP488 inhibits binding of multiple RAGE ligands, including AGEs, HMGB1, S100B, and Aβ. In a mouse model of AD, TTP488 administration inhibited inflammatory signaling, neuronal Aβ accumulation, and neurocognitive function (6, 88–90). A phase II double-blind trial in mild to moderate AD focused on the safety and tolerability profile of TTP488 (88). In 2007, a larger 18-month phase II trial assigned 399 mild to moderate AD subjects (NCT00566397) to a low-dose, high-dose, or placebo group (6, 89). The study was discontinued at six months as the high-dose group displayed worsening cognitive measures (6, 89). However, follow-up analysis of the low-dose group showed a clinical benefit of slowing cognitive decline (6). As a result, a phase III trial (NCT02080364, the STEADFAST Study) is currently under way in a mild AD cohort of 800 subjects and should be completed in 2017 (6).

Various groups have developed new TTP488 derivatives by modifying the imidazole ring, the hydrophobic side groups, and the aromatic core (91, 92). These studies have shown the aminoalkyl linker is necessary for effective inhibition of RAGE ligand binding and signaling. Most studies testing derivatives of TTP488 have been performed in preclinical models of AD, where inhibition of disease was seen (91, 92). None of these derivatives have progressed to human clinical studies to date.

FPS-ZM1. To generate a novel class of RAGE inhibitors, Deane et al. (7) screened a library of 5,000 small molecules for their ability to inhibit interaction of RAGE-Aβ. Once lead compounds were identified, a second-generation library of 100 structurally related compounds was screened for RAGE-Aβ inhibition and ability to cross the BBB. A number of candidates were then tested. A compound named FPS-ZM1 blocked inflammatory signaling in the mouse brain, reduced Aβ accumulation, and improved cognitive performance. Importantly, FPS-ZM1 did not cause toxic side effects in mice, even at doses as high as 500 mg/kg (7).

FPS-ZM1 has been explored in other mouse models of neuropathology (71, 93–98). Using a rat model of neuroinflammation with intrahippocampal injection of AGEs, FPS-ZM1 reduced brain inflammation and Aβ production and improved cognitive function (96). In a rat stroke model of intracerebral hemorrhage, FPS-ZM1 reduced the BBB dysfunction and nerve fiber injury associated with early brain injury after stroke (94, 95). In primary cultured rat microglia, FPS-ZM1 impaired AGE-induced inflammation and reactive oxygen species (93). In heart failure induced by transverse aortic constriction in mice, eight weeks of FPS-ZM1 administration inhibited cardiac hypertrophy and reduced inflammation in cardiac tissues (97). In a mouse model of asthma induced by toluene diisocyanate, FPS-ZM1 administration impaired airway reactivity, inflammation, and aberrant β-catenin signaling (98).

Most recently, our group has demonstrated that small-molecule inhibitors of RAGE including FPS-ZM1 impair cancer progression and metastasis (71). In vitro studies with highly metastatic breast cancer cells revealed that FPS-ZM1 abrogated the excess invasion caused by RAGE. No effect was seen on cell viability with FPS-ZM1, suggesting that inhibiting RAGE with FPS-ZM1 affects its migratory and invasive properties. In a mouse xenograft model, intraperitoneal injection of FPS-ZM1 or vehicle control impaired tumor growth and metastasis to lung and liver, compared to vehicle treated controls (71).

Intracellular Inhibitors of RAGE

As the intracellular region of RAGE is critical in engaging signaling partners and inducing downstream effects (15, 56), small-molecule inhibitors were identified that interfere with RAGE–mDia1

interaction (99). Lead compounds inhibit AGE–RAGE proinflammatory signaling and decrease cardiac injury in an ischemic mouse model (99). Further work is needed in other cell systems and in vivo models to test whether these compounds are effective therapeutics.

CONCLUSIONS

RAGE is clearly an important molecule in the innate immune system and in propagating the inflammatory response to a variety of endogenous DAMPs and exogenous PAMPs. As a result, RAGE has been implicated in major inflammatory disease states including diabetes, CVD, cancer, and neurological disease. Animal models of these disease states show that therapeutically targeting RAGE inhibits chronic disease. Studies are required to translate these promising findings to human clinical trials now that small-molecule inhibitors of RAGE have been shown to be well tolerated in humans. Targeting RAGE, with complementary combinational approaches, could yield novel therapies for chronic inflammatory disease.

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LITERATURE CITED

1. Neeper M, Schmidt AM, Brett J, et al. 1992. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J. Biol. Chem.* 267:14998–5004
2. **Chavakis T, Bierhaus A, Al-Fakhri N, et al. 2003. The pattern recognition receptor (RAGE) is a counterreceptor for leukocyte integrins: a novel pathway for inflammatory cell recruitment. *J. Exp. Med.* 198:1507–15**
3. Kalea AZ, Schmidt AM, Hudson BI. 2009. RAGE: a novel biological and genetic marker for vascular disease. *Clin. Sci.* 116:621–37
4. Kierdorf K, Fritz G. 2013. RAGE regulation and signaling in inflammation and beyond. *J. Leukoc. Biol.* 94:55–68
5. Constien R, Forde A, Liliensiek B, et al. 2001. Characterization of a novel EGFP reporter mouse to monitor Cre recombination as demonstrated by a Tie2 Cre mouse line. *Genesis* 30:36–44
6. Burstein AH, Grimes I, Galasko DR, et al. 2014. Effect of TTP488 in patients with mild to moderate Alzheimer's disease. *BMC Neurol.* 14:12
7. Deane R, Singh I, Sagare AP, et al. 2012. A multimodal RAGE-specific inhibitor reduces amyloid beta-mediated brain disorder in a mouse model of Alzheimer disease. *J. Clin. Investig.* 122:1377–92
8. Brownlee M. 1995. Advanced protein glycosylation in diabetes and aging. *Annu. Rev. Med.* 46:223–34
9. Trivelli L, Ranney HM, Lai HTL. 1971. Hemoglobin components in patients with diabetes mellitus. *N. Engl. J. Med.* 284:353–57
10. **The Diabetes Control and Complications Trial Research Group. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* 329:977–86**
11. Stratton IM, Adler AI, Neil HA, et al. 2001. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321:405–12

2. This paper defined RAGE as a PRR.

10. The DCCT was a landmark study that clearly linked control of glucose and glycation to diabetic vascular complications.

12. Thornalley PJ, Langborg A, Minhas HS. 1999. Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose. *Biochem. J.* 344:109–16
13. Kislinger T, Fu C, Huber C, et al. 1999. N(epsilon)-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signaling pathways and modulate gene expression. *J. Biol. Chem.* 274:31740–49
14. Fritz G. 2011. RAGE: a single receptor fits multiple ligands. *Trends Biochem. Sci.* 36:625–32
15. Hudson BI, Kalea AZ, Arriero MD, et al. 2008. Interaction of the RAGE cytoplasmic domain with diaphanous-1 is required for ligand-stimulated cellular migration through activation of Rac1 and Cdc42. *J. Biol. Chem.* 283:34457–68
16. Sessa L, Gatti E, Zeni F, et al. 2014. The receptor for advanced glycation end-products (RAGE) is only present in mammals, and belongs to a family of cell adhesion molecules (CAMs). *PLOS ONE* 9:e86903
17. Cavallaro U, Dejana E. 2011. Adhesion molecule signalling: not always a sticky business. *Nat. Rev. Mol. Cell Biol.* 12:189–97
18. Sugaya K, Fukagawa T, Matsumoto KI, et al. 1994. Three genes in the human MHC class III region near the junction with the class II: gene for receptor of advanced glycosylation end products, PBX2 homeobox gene and a notch homolog, human counterpart of mouse mammary tumor gene *int*-3. *Genomics* 23:408–19
19. Hudson BI, Carter AM, Harja E, et al. 2008. Identification, classification, and expression of RAGE gene splice variants. *FASEB J.* 22:1572–80
20. Kalea AZ, Reiniger N, Yang H, et al. 2009. Alternative splicing of the murine receptor for advanced glycation end-products (RAGE) gene. *FASEB J.* 23:1766–74
21. Fehrenbach H, Kasper M, Tschernig T, et al. 1998. Receptor for advanced glycation endproducts (RAGE) exhibits highly differential cellular and subcellular localisation in rat and human lung. *Cell Mol. Biol.* 44:1147–57
22. **Hori O, Brett J, Slattery T, et al. 1995. The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of RAGE and amphoterin in the developing nervous system. *J. Biol. Chem.* 270:25752–61**
23. Yan SD, Chen X, Fu J, et al. 1996. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. *Nature* 382:685–91
24. Hofmann MA, Drury S, Fu CF, et al. 1999. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97:889–901
25. Suresh R, Mosser DM. 2013. Pattern recognition receptors in innate immunity, host defense, and immunopathology. *Adv. Physiol. Educ.* 37:284–91
26. Scaffidi P, Misteli T, Bianchi ME. 2002. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 418:191–95
27. Kang R, Zhang Q, Zeh HJ III, et al. 2013. HMGB1 in cancer: good, bad, or both? *Clin. Cancer Res.* 19:4046–57
28. Wang H, Bloom O, Zhang M, et al. 1999. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 285:248–51
29. Donato R. 2001. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int. J. Biochem. Cell Biol.* 33:637–68
30. Leclerc E, Fritz G, Vetter SW, Heizmann CW. 2009. Binding of S100 proteins to RAGE: an update. *Biochim. Biophys. Acta* 1793:993–1007
31. Ostendorp T, Leclerc E, Galichet A, et al. 2007. Structural and functional insights into RAGE activation by multimeric S100B. *EMBO J.* 26:3868–78
32. Perera C, McNeil HP, Geczy CL. 2010. S100 Calgranulins in inflammatory arthritis. *Immunol. Cell Biol.* 88:41–49
33. Rammes A, Roth J, Goebeler M, et al. 1997. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J. Biol. Chem.* 272:9496–502
34. Bjarnason I. 2017. The use of fecal calprotectin in inflammatory bowel disease. *Gastroenterol. Hepatol.* 13:53–56
35. Deane R, Du YS, Submamaryan RK, et al. 2003. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nat. Med.* 9:907–13

22. Demonstrated that RAGE can bind other ligands than AGEs.

36. Sirois CM, Jin T, Miller AL, et al. 2013. RAGE is a nucleic acid receptor that promotes inflammatory responses to DNA. *J. Exp. Med.* 210:2447–63
37. Liu L, Yang M, Kang R, et al. 2014. HMGB1-DNA complex-induced autophagy limits AIM2 inflammasome activation through RAGE. *Biochem. Biophys. Res. Commun.* 450:851–56
38. Yamamoto Y, Harashima A, Saito H, et al. 2011. Septic shock is associated with receptor for advanced glycation end products ligation of LPS. *J. Immunol.* 186:3248–57
39. Tian J, Huang K, Krishnan S, et al. 2013. RAGE inhibits human respiratory syncytial virus syncytium formation by interfering with F-protein function. *J. Gen. Virol.* 94:1691–700
40. Anisuzzaman, Hatta T, Miyoshi T, et al. 2014. Longistatin in tick saliva blocks advanced glycation end-product receptor activation. *J. Clin. Investig.* 124:4429–44
41. Uribarri J, del Castillo MD, de la Maza MP, et al. 2015. Dietary advanced glycation end products and their role in health and disease. *Adv. Nutr.* 6:461–73
42. Stirban A, Negrean M, Gotting C, et al. 2008. Dietary advanced glycation endproducts and oxidative stress: in vivo effects on endothelial function and adipokines. *Ann. N. Y. Acad. Sci.* 1126:276–79
43. Poulsen MW, Andersen JM, Hedegaard RV, et al. 2016. Short-term effects of dietary advanced glycation end products in rats. *Br. J. Nutr.* 115:629–36
44. Koch M, Chitayat S, Dattilo BM, et al. 2010. Structural basis for ligand recognition and activation of RAGE. *Structure* 18:1342–52
45. Leclerc E, Fritz G, Weibel M, et al. 2007. S100B and S100A6 differentially modulate cell survival by interacting with distinct RAGE (receptor for advanced glycation end products) immunoglobulin domains. *J. Biol. Chem.* 282:31317–31
46. Yatime L, Betzer C, Jensen RK, et al. 2016. The structure of the RAGE:S100A6 complex reveals a unique mode of homodimerization for S100 proteins. *Structure* 24:2043–52
47. Zong H, Madden A, Ward M, et al. 2010. Homodimerization is essential for the receptor for advanced glycation end products (RAGE)-mediated signal transduction. *J. Biol. Chem.* 285:23137–46
48. Yatime L, Andersen GR. 2013. Structural insights into the oligomerization mode of the human receptor for advanced glycation end-products. *FEBS J.* 280:6556–68
49. Rouhiainen A, Kuja-Panula J, Tumova S, Rauvala H. 2013. RAGE-mediated cell signaling. *Methods Mol. Biol.* 963:239–63
50. Sakaguchi T, Yan SF, Yan SD, et al. 2003. Central role of RAGE-dependent neointimal expansion in arterial restenosis. *J. Clin. Investig.* 111:959–72
51. Taguchi A, Blood DC, del Toro G, et al. 2000. Blockage of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 405:354–60
52. Jules J, Miguel D, Hudson BI. 2013. Alternative splicing of the RAGE cytoplasmic domain regulates cell signaling and function. *PLOS ONE* 8:e78267
53. Ishihara K, Tsutsumi K, Kawane S, et al. 2003. The receptor for advanced glycation end-products (RAGE) directly binds to ERK by a D-domain-like docking site. *FEBS Lett.* 550:107–13
54. Sakaguchi M, Murata H, Yamamoto K, et al. 2011. TIRAP, an adaptor protein for TLR2/4, transduces a signal from RAGE phosphorylated upon ligand binding. *PLOS ONE* 6:e23132
55. Yamamoto K, Murata H, Putranto EW, et al. 2013. DOCK7 is a critical regulator of the RAGE-Cdc42 signaling axis that induces formation of dendritic pseudopodia in human cancer cells. *Oncol. Rep.* 29:1073–79
56. Rai V, Maldonado AY, Burz DS, et al. 2012. Signal transduction in receptor for advanced glycation end products (RAGE): solution structure of C-terminal RAGE (ctRAGE) and its binding to mDia1. *J. Biol. Chem.* 287:5133–44
57. Wautier JL, Zoukourian C, Chappey O, et al. 1996. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy. Soluble receptor for advanced glycation end products blocks hyperpermeability in diabetic rats. *J. Clin. Investig.* 97:238–43
58. Park L, Raman KG, Lee KJ, et al. 1997. A murine model of accelerated diabetic atherosclerosis: suppression by soluble receptor for advanced glycation endproducts. *Circulation* 96:3079
59. Wendt T, Harja E, Bucciarelli L, et al. 2006. RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes. *Atherosclerosis* 185:70–77

60. Soro-Paavonen A, Watson AM, Li J, et al. 2008. Receptor for advanced glycation end products (RAGE) deficiency attenuates the development of atherosclerosis in diabetes. *Diabetes* 57:2461–69
61. Harja E, Bu DX, Hudson BI, et al. 2008. Vascular and inflammatory stresses mediate atherosclerosis via RAGE and its ligands in apoE–/– mice. *J. Clin. Investig.* 118:183–94
62. Bucciarelli LG, Kaneko M, Ananthakrishnan R, et al. 2006. Receptor for advanced-glycation end products: key modulator of myocardial ischemic injury. *Circulation* 113:1226–34
63. Tanji N, Markowitz GS, Fu CF, et al. 2000. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. *J. Am. Soc. Nephrol.* 11:1656–66
64. Wendt TM, Tanji N, Guo J, et al. 2003. RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am. J. Pathol.* 162:1123–37
65. Barile GR, Pachydaki SI, Tari SR, et al. 2005. The RAGE axis in early diabetic retinopathy. *Investig. Ophthalmol. Vis. Sci.* 46:2916–24
66. Toth C, Rong LL, Yang C, et al. 2008. Receptor for advanced glycation end products (RAGEs) and experimental diabetic neuropathy. *Diabetes* 57:1002–17
67. Goova MT, Li J, Kislinger T, et al. 2001. Blockade of receptor for advanced glycation end-products restores effective wound healing in diabetic mice. *Am. J. Pathol.* 159:513–25
68. Juranek J, Ray R, Banach M, Rai V. 2015. Receptor for advanced glycation end-products in neurodegenerative diseases. *Rev. Neurosci.* 26:691–98
69. Kang R, Tang D, Schapiro NE, et al. 2010. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. *Cell Death. Differ.* 17:666–76
70. Logsdon CD, Fuentes MK, Huang EH, Arumugam T. 2007. RAGE and RAGE ligands in cancer. *Curr. Mol. Med.* 7:777–89
71. Kwak T, Drews-Elger K, Ergonul A, et al. 2017. Targeting of RAGE-ligand signaling impairs breast cancer cell invasion and metastasis. *Oncogene* 36:1559–72
72. Kalea AZ, See F, Harja E, et al. 2010. Alternatively spliced RAGEv1 inhibits tumorigenesis through suppression of JNK signaling. *Cancer Res.* 70:5628–38
73. Hsieh HL, Schafer BW, Sasaki N, Heizmann CW. 2003. Expression analysis of S100 proteins and RAGE in human tumors using tissue microarrays. *Biochem. Biophys. Res. Commun.* 307:375–81
74. Ishiguro H, Nakaigawa N, Miyoshi Y, et al. 2005. Receptor for advanced glycation end products (RAGE) and its ligand, amphoterin are overexpressed and associated with prostate cancer development. *Prostate* 64:92–100
75. Kuniyasu H, Chihara Y, Takahashi T. 2003. Co-expression of receptor for advanced glycation end products and the ligand amphoterin associates closely with metastasis of colorectal cancer. *Oncol. Rep.* 10:445–48
76. Kuniyasu H, Oue N, Wakikawa A, et al. 2002. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. *J. Pathol.* 196:163–70
77. Turovskaya O, Foell D, Sinha P, et al. 2008. RAGE, carboxylated glycans and S100A8/A9 play essential roles in colitis-associated carcinogenesis. *Carcinogenesis* 29:2035–43
78. Vernon PJ, Loux TJ, Schapiro NE, et al. 2013. The receptor for advanced glycation end products promotes pancreatic carcinogenesis and accumulation of myeloid-derived suppressor cells. *J. Immunol.* 190:1372–79
79. Gebhardt C, Riehl A, Durchdewald M, et al. 2008. RAGE signaling sustains inflammation and promotes tumor development. *J. Exp. Med.* 205:275–85
80. Boyapati RK, Rossi AG, Satsangi J, Ho GT. 2016. Gut mucosal DAMPs in IBD: from mechanisms to therapeutic implications. *Mucosal. Immunol.* 9:567–82
81. Hofmann MA, Drury S, Hudson BI, et al. 2003. RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immun.* 3:123–35
82. Hancock DB, Eijgelsheim M, Wilk JB, et al. 2010. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat. Genet.* 42:45–52
83. Repapi E, Sayers I, Wain LV, et al. 2010. Genome-wide association study identifies five loci associated with lung function. *Nat. Genet.* 42:36–44

84. Al-Robaiy S, Weber B, Simm A, et al. 2013. The receptor for advanced glycation end-products supports lung tissue biomechanics. *Am. J. Physiol. Lung Cell Mol. Physiol.* 305:L491–L500
85. Yonekura H, Yamamoto Y, Sakurai S, et al. 2003. Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem. J.* 370:1097–109
86. Zhang L, Bukulin M, Kojro E, et al. 2008. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J. Biol. Chem.* 283:35507–16
87. Falcone C, Emanuele E, D'Angelo A, et al. 2005. Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. *Arterioscler. Thromb. Vasc. Biol.* 25:1032–37
88. Sabbagh MN, Agro A, Bell J, et al. 2011. PF-04494700, an oral inhibitor of receptor for advanced glycation end products (RAGE), in Alzheimer disease. *Alzheimer Dis. Assoc. Disord.* 25:206–12
89. Galasko D, Bell J, Mancuso JY, et al. 2014. Clinical trial of an inhibitor of RAGE-Aβ interactions in Alzheimer disease. *Neurology* 82:1536–42
90. Kostura MA, Kindy M, Burstein AH, et al. 2014. Efficacy of RAGE antagonists in murine models of Alzheimer's disease. *Alzheimer's Dement.* 10(4):P638
91. Lee YS, Kim H, Kim YH, et al. 2012. Synthesis and structure-activity relationships of tri-substituted thiazoles as RAGE antagonists for the treatment of Alzheimer's disease. *Bioorg. Med. Chem. Lett.* 22:7555–61
92. Han YT, Choi GI, Son D, et al. 2012. Ligand-based design, synthesis, and biological evaluation of 2-aminopyrimidines, a novel series of receptor for advanced glycation end products (RAGE) inhibitors. *J. Med. Chem.* 55:9120–35
93. Shen C, Ma Y, Zeng Z, et al. 2017. RAGE-specific inhibitor FPS-ZM1 attenuates AGEs-induced neuroinflammation and oxidative stress in rat primary microglia. *Neurochem. Res.* 42(10):2902–11
94. Li D, Lei C, Zhang S, et al. 2015. Blockade of high mobility group box-1 signaling via the receptor for advanced glycation end-products ameliorates inflammatory damage after acute intracerebral hemorrhage. *Neurosci. Lett.* 609:109–19
95. Yang F, Wang Z, Zhang JH, et al. 2015. Receptor for advanced glycation end-product antagonist reduces blood-brain barrier damage after intracerebral hemorrhage. *Stroke* 46:1328–36
96. Hong Y, Shen C, Yin Q, et al. 2016. Effects of RAGE-specific inhibitor FPS-ZM1 on amyloid-β metabolism and AGEs-induced inflammation and oxidative stress in rat hippocampus. *Neurochem. Res.* 41:1192–99
97. Liu Y, Yu M, Zhang Z, et al. 2016. Blockade of receptor for advanced glycation end products protects against systolic overload-induced heart failure after transverse aortic constriction in mice. *Eur. J. Pharmacol.* 791:535–43
98. Yao L, Zhao H, Tang H, et al. 2016. The receptor for advanced glycation end products is required for beta-catenin stabilization in a chemical-induced asthma model. *Br. J. Pharmacol.* 173:2600–13
99. Manigrasso MB, Pan J, Rai V, et al. 2016. Small molecule inhibition of ligand-stimulated RAGE-DIAPH1 signal transduction. *Sci. Rep.* 6:22450

87. The use of serum sRAGE levels as a biomarker for clinical disease was a critical step in the understanding of RAGE in human disease.