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The Receptor for Advanced Glycation End Products (RAGE) and DIAPH1: Implications for vascular and neuroinflammatory dysfunction in disorders of the central nervous system

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A B S T R A C T

The Receptor for Advanced Glycation End Products (RAGE) is expressed by multiple cell types in the brain and spinal cord that are linked to the pathogenesis of neurovascular and neurodegenerative disorders, including neurons, glia (microglia and astrocytes) and vascular cells (endothelial cells, smooth muscle cells and pericytes). Mounting structural and functional evidence implicates the interaction of the RAGE cytoplasmic domain with the formin, Diaphanous1 (DIAPH1), as the key cytoplasmic hub for RAGE ligand-mediated activation of cellular signaling. In aging and diabetes, the ligands of the receptor abound, both in the central nervous system (CNS) and in the periphery. Such accumulation of RAGE ligands triggers multiple downstream events, including upregulation of RAGE itself. Once set in motion, cell intrinsic and cell-cell communication mechanisms, at least in part via RAGE, trigger dysfunction in the CNS. A key outcome of endothelial dysfunction is reduction in cerebral blood flow and increased permeability of the blood brain barrier, conditions that facilitate entry of activated leukocytes into the CNS, thereby amplifying primary nodes of CNS cellular stress. This contribution details a review of the ligands of RAGE, the mechanisms and consequences of RAGE signal transduction, and cites multiple examples of published work in which RAGE contributes to the pathogenesis of neurovascular perturbation. Insights into potential therapeutic modalities targeting the RAGE signal transduction axis for disorders of CNS vascular dysfunction and neurodegeneration are also discussed.

1. Introduction

The Receptor for Advanced Glycation End Products (RAGE) was discovered on account of its ability to bind the products of non-enzymatic glycation and oxidation of proteins/lipids, termed advanced glycation end products (AGEs). In addition to AGEs, RAGE binds a number of distinct ligands, such as those involved in immune/inflammatory responses, such as S100/calgranulins and high mobility group box 1 (HMGB1), and oligomeric forms of amyloid-β peptide (Aβ) (Lopez-Diez et al., 2016; Ramasamy et al., 2016). The consequent discovery that RAGE was a multi-ligand receptor set the stage for a fuller understanding of the biology of RAGE in homeostasis and in disease.

Although the highest expression of RAGE in homeostatic states is in the lung, a plethora of evidence suggests that in both human subjects and in experimental model systems, the expression of the receptor is enhanced in settings in which its ligands become more abundant. For example, in tissues affected by diabetes, aging, neurodegeneration, ischemia/reperfusion injury (Aleshin et al., 2008; Bucciarelli et al., 2006, 2008), chronic inflammation and cancer, higher expression of RAGE has been observed compared to non-affected control tissues (Cipollone et al., 2003; Juranek et al., 2015; Palanissami and Paul, 2018; Yan et al., 1996). RAGE is expressed on multiples different cell types, including vascular cells, such as endothelial cells (ECs) and smooth muscle cells (SMCs); and immune/inflammatory cells, such as neutrophils, monocytes/macrophages, T and B lymphocytes and dendritic cells (Avalos et al., 2010; Daffu et al., 2015; Dumitriu et al., 2005; Moser et al., 2007; Wautier et al., 1996). Vascular and immune cell dysfunction exacerbates cellular homeostasis in RAGE-expressing target cells in chronic diseases, such as neurons, cardiomyocytes, glomerular epithelial cells (podocytes) and skeletal muscle (Shang et al., 2010; Sorci et al., 2003; Tanji et al., 2000; Yan et al., 1996).

The fact that RAGE is differentially expressed in various organs and cell types suggested RAGE-dependent and -independent cell intrinsic and/or cell-cell communication networks in homeostasis and disease. This review highlights the role of RAGE in the central nervous system (CNS) and details how vascular, immune cell, and neuronal interactions might play important roles in disease pathogenesis and progression, and how the temporal cues of such cell intrinsic and cell-cell networking vis-à-vis RAGE may mediate pro-repair and survival vs. pro-inflammatory and anti-repair signals, leading to cell death. Finally, this review will provide insight into novel therapeutic approaches targeting RAGE signaling.

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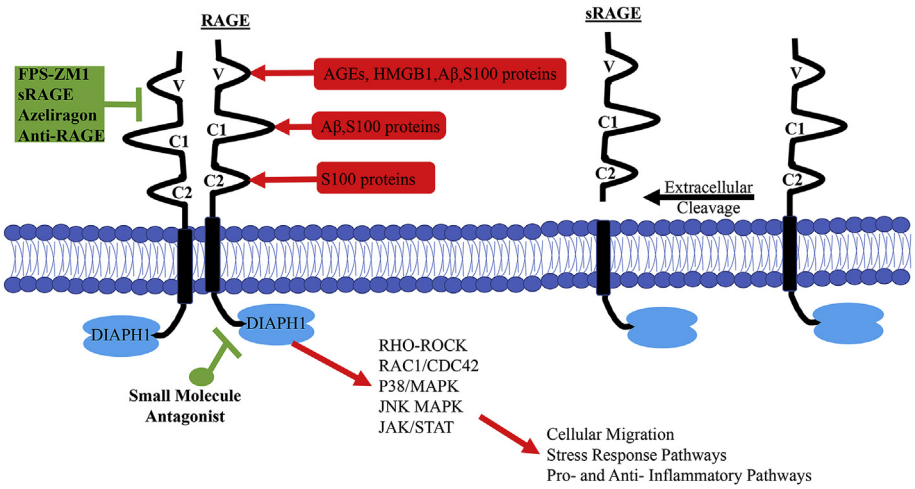

Fig. 1. Schematic of RAGE signal transduction and examples of inhibition by antagonists. The extracellular immunoglobin-like (Ig) domains of RAGE (V, C1, C2) all have been shown to bind RAGE ligands. Many widely-used receptor antagonists (Anti-RAGE antibodies, Azeliragon, and FPS-ZM1) interact with the extracellular domains to prevent ligand engagement. Soluble RAGE (sRAGE) is postulated to sequester RAGE ligands and prevent their engagement with the dimerized full-length receptor. There are two forms of sRAGE: the first is produced by extracellular proteolytic cleavage of the full-length receptor, which liberates the extracellular domains from the transmembrane domain and intracellular domain, and the second is a product of an mRNA splice variant. The formin, Diaphanous-1 (DIAPH1), interacts with the cytoplasmic tail of RAGE and mediates signal transduction. Several candidate small molecule antagonists have been generated to prevent the RAGE-DIAPH1 interaction. RAGE signal transduction is cell-type dependent but can include RHO/ROCK, RAC1/CDC42, P38/MAPK, JNK MAPK and JAK/STAT. These events result in changes to cellular migration, stress responses, and regulation of pro- and anti- inflammatory pathways (NFκB activation) as well as other changes in cellular properties.

2. RAGE structure and soluble forms

RAGE is a member of the immunoglobulin (Ig) superfamily of cell surface molecules. It is composed of three extracellular domains (one V-type and two C-type Ig-like domains). These extracellular domains are followed by a single, hydrophobic transmembrane domain and by a short, less than 45 amino acid cytoplasmic domain, which is essential for RAGE ligand-mediated signaling (Fig. 1). The extracellular domains of RAGE are the sites of known extracellular ligand binding. Although the V-type domain is the preferential binding site for most of the ligand families, other reports have indicated that the C-type domains may also mediate binding of some of the RAGE ligands. The V-type Ig domain itself is heterogeneous, as distinct binding pockets, characterized by their charge or hydrophobicity states, exist on this domain for ligand engagement (Xie et al., 2007, 2008).

The extracellular domains of RAGE, known as soluble RAGE, may exist in soluble form and in *in vitro* and *in vivo* experimentation, appear to sequester RAGE ligands and block their binding to and activation of cell surface receptors, including membrane bound RAGE (Schmidt et al., 1994). There are two forms of soluble RAGEs that have been identified in human subjects (Schmidt, 2015). The first is cell surface-cleaved soluble or sRAGE, which is produced via the actions of metalloproteases or other molecules, such as ADAM-10 (a disintegrin and metalloproteinase domain-containing protein 10) (Raucci et al., 2008), and the second form, called endogenous secretory or esRAGE, is the result of an RNA splice variant (Yonekura et al., 2003). Research tools have been employed to measure total sRAGEs (including cell surface cleaved sRAGE and esRAGE) or esRAGE alone on human subject serum/plasma or other tissue fluids (such as cerebrospinal fluid (CSF)) to assess possible relationships to the state and/or the extent of disease. For example, in diabetes, aging, stroke, subarachnoid hemorrhage (SAH), and in neurodegenerative disorders, such as Alzheimer's Disease (AD) or amyotrophic lateral sclerosis (ALS), measurements of sRAGEs have been reported to distinguish affected vs. unaffected subjects (Emanuele et al., 2005; Ilzecka, 2009; Loomis et al., 2017; Lue et al., 2009; Saito et al., 2017; Sokol et al., 2017; Tang et al., 2017). The extent to which measurements of sRAGEs may serve as reliable and reproducible biomarkers of RAGE-related chronic diseases, however, is uncertain. Given the multiple settings in which RAGE appears to be related to pathobiology, the specificity/sensitivity of soluble RAGEs as a biomarker for disease onset, progression and/or the response to therapeutic intervention, is uncertain and remains to be tested.

3. RAGE and mechanisms of signal transduction

The cytoplasmic domain of RAGE lacks endogenous kinase activity and through a yeast two-hybrid assay, it was discovered that this domain of RAGE bound the FH1 (formin homology 1) domain of Diaphanous1 (DIAPH1) (Hudson et al., 2008). Formins such as DIAPH1 play key roles in actin cytoskeleton rearrangements and regulation of Rho GTPases and serum response factor (SRF)-dependent genes, factors and pathways that regulate cellular migration, signal transduction and stress-responsive gene programs (Chesarone et al., 2010; Kühn and Geyer, 2014). Collectively, these properties have been shown to be key consequences of ligand-RAGE signaling. Multiple studies in SMCs, ECs, macrophages, and cardiomyocytes, for example, indicate that DIAPH1 is required for the effects of RAGE ligands to modulate signal transduction and functional outcomes in these cells (Hudson et al., 2008; Touré et al., 2012; Xu et al., 2010; Zhou et al., 2018). *In vivo*, deletion of *Diaph1* protects from cardiac ischemia-reperfusion injury and hypoxia-related upregulation of *Egr1,* which encodes a transcriptional regulator protein highly expressed in the CNS, such as in microglia, (Landis et al., 1993); diabetes-associated pathologies in the kidney; macrophage inflammation; and restenosis after endothelial denudation of the femoral artery (Manigrasso et al., 2018; O'Shea et al., 2017; Touré et al., 2012; Xu et al., 2010), all phenotypes that parallel the observations in *Ager* (the gene encoding RAGE) null mice in these stress conditions.

Recent work has solved the structure of the RAGE cytoplasmic domain and the nature of its interaction with DIAPH1 and has revealed that mutation of RAGE cytoplasmic domain R5/Q6 amino acid residues to alanine residues resulted in failure to bind DIAPH1 by NMR spectroscopy and failure of RAGE ligand-mediated signal transduction in cultured SMCs (Rai et al., 2012; Xue et al., 2016).

In the section to follow, this review will consider how systemic conditions may modulate the levels of RAGE ligands and RAGE, and, thereby, affect the functions of multiple cell types in the CNS, including those associated with the blood-brain barrier (BBB).

4. Systemic disorders & modulation of the BBB: A spark that ignites CNS dysfunction

The BBB is composed of a continuous monolayer of ECs with closely apposed astrocytic foot processes and pericytes embedded into the nonfenestrated basement membrane of the border. These ECs are connected by tight junctions, which function to regulate entry of large macromolecules, cells and pathogens. Although oxygen and carbon dioxide flow freely, the BBB is responsible for the exclusion of the entry

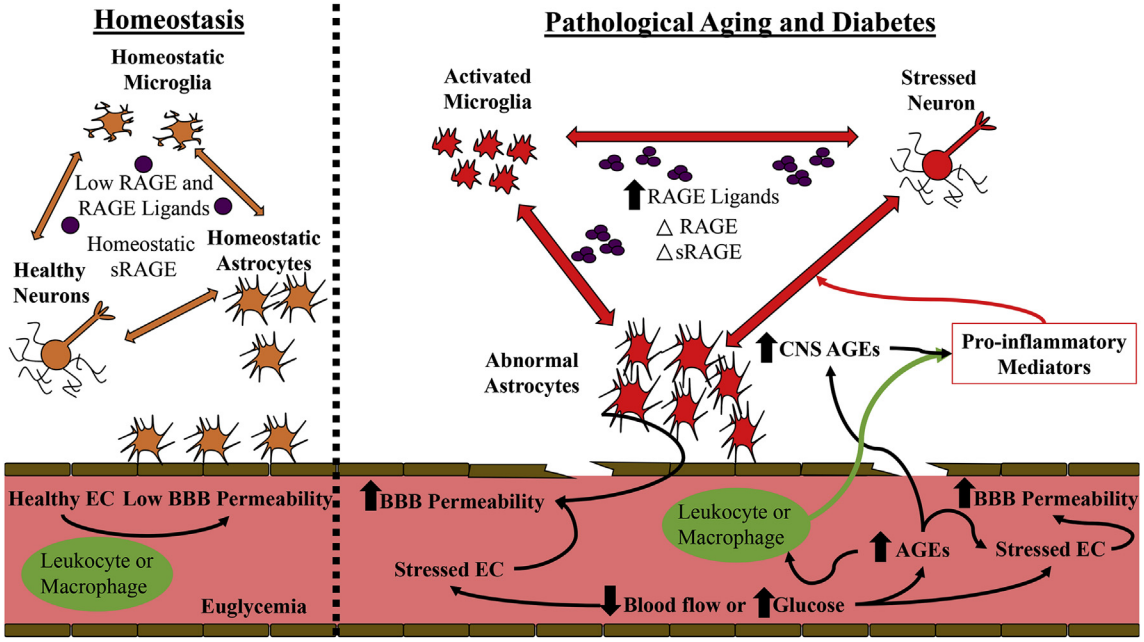

Fig. 2. Working model of RAGE-dependent contributions to blood-brain barrier (BBB) dysfunction. Increases in RAGE ligands, blood glucose and/or decreased blood flow promote the transition from homeostasis to disease by promoting BBB dysfunction, AGE generation, and overall cellular stress within neurons, microglia, astrocytes and endothelial cells (EC). Depending on the disease and aging condition, RAGE and sRAGE levels may be altered. We posit that age-, diabetes-, or neurodegenerative disease- induced AGE accumulation within the CNS and the periphery initiates and perpetuates endothelial permeability and BBB dysfunction, thereby promoting BBB breakdown, at least in part via RAGE signaling.

of species that might cause harm to the vulnerable brain and spinal cord (Iadecola, 2017; Kisler et al., 2017; Presta et al., 2018) and for modulating blood flow appropriately in various locations during activities in which metabolic and oxygenation requirements are altered. Two conditions in which the BBB becomes gradually more compromised include diabetes and natural aging, both known risk factors for neurodegenerative disorders such as AD and other neurodegenerative disorders (Sweeney et al., 2018) and for ischemic stroke; all settings in which RAGE ligands accumulate and are plentiful (Fig. 2).

4.1. Diabetes

Glucose is essential for brain metabolism and is normally transported into the brain by specialized transporters (GLUT). However, in excess, glucose may *directly* activate pathways such as Protein Kinase C and the polyol pathway, thereby activating mechanisms that promote oxidative stress and inflammation, such as in resident microglia. Further, if the BBB is compromised, the influx of periphery-derived immune/inflammatory cells may ensue, thereby augmenting inflammation. In addition, high glucose may upregulate levels of hypoxia-inducible factor-1α (HIF-1α), thereby leading to increased expression of Vascular Endothelial Growth Factor (VEGF) and, consequently, increased vascular permeability (Van Dyken and Lacoste, 2018).

In addition to such potential direct effects of high levels of glucose, increased glucose levels may result in the generation of AGEs. AGEs, via their interactions with their central cell surface receptor, RAGE, may result in amplification of inflammatory and oxidative stress, at least in part through the activation of NF-κB. In streptozotocin-induced type 1 diabetic mice, expression of RAGE was shown to be increased at the BBB (Liu et al., 2009). In non-CNS vascular beds, in diabetic rats, AGEs mediated vascular permeability in a RAGE-dependent manner and in coronary arterioles of type 2 diabetic mice, endothelial dysfunction was reversed by treatment with sRAGE (Gao et al., 2008; Wautier et al., 1996), thus suggesting that sequestration of RAGE ligands and suppression of RAGE/DIAPH1 signaling is beneficial for overall homeostasis and BBB integrity.

4.2. Aging

It is well-established that aging is the chief risk factor for vascular disorders of the brain and for neurodegenerative processes. Reduced blood flow, disruption of microvascular integrity and BBB dysfunction accompany aging (Farkas and Luiten, 2001; Montagne et al., 2015; Park et al., 2018). Recent work, using CSF biomarkers and dynamic contrast-enhanced magnetic resonance imaging in human subjects, has shown that breakdown of the BBB around the hippocampus is a very early marker of cognitive impairment and that these pathologies are independent of Aβ deposition or tau aggregation (Nation et al., 2019). In this context, AGEs increase even in euglycemic aging, based on the nonenzymatic glycation/oxidation of long-lived proteins whose exposure to even normal levels of glucose may, ultimately, result in formation of AGE adducts. In the aortas of aged Fischer 344 rats, the concentration of the key AGE precursor, methylglyoxal (MG), was significantly increased compared with aortas from young Fischer rats. Impaired aging-associated endothelial-dependent relaxation in aortic rings from aged animals was reversed by treatment with sRAGE (Hallam et al., 2010).

Collectively, these considerations suggest that one of the components of BBB dysfunction in key settings linked to neurodegeneration and vascular damage to the CNS is the ligand-RAGE pathway. The extent to which DIAPH1 may, or may not, contribute to the maintenance or dysfunction of the BBB remains to be determined. However, it is conceivable that DIAPH1 perturbation may contribute importantly to endothelial dysfunction and loss of BBB integrity given the roles of this molecule in actin cytoskeleton organization and the observation that ligand stimulation of the RAGE/DIAPH1 axis generates oxidative stress, through NADPH oxidases, and promotes inflammation (Touré et al., 2012). Recent work showed that DIAPH1 is expressed in human aged AD brain to a greater extent than that in age-matched controls and in multiple cell types (Derk et al., 2018a). In the sections to follow, this review will consider settings of CNS dysfunction in which roles for the RAGE axis have been postulated and probed.

5. CNS vascular disorders and the RAGE axis

In human subjects, investigators have studied whether genetic variations in *AGER* might contribute to risk of various forms of vascular disorders in the CNS. In the Sahlgrenska Academy Study on Ischaemic Stroke, 732 Caucasian subjects with first-ever and 112 Caucasian subjects with recurrent ischemic stroke were compared with 668 Caucasian controls. Of three tested *AGER* single nucleotide polymorphisms (SNP), one SNP, rs1035798 (maps to the third *AGER* intron), showed a significant association with small vessel disease subtype of stroke, which was independent of hypertension, diabetes and smoking. However, none of these SNPs demonstrated significant associations with overall ischemic stroke (Olsson and Jood, 2013). In a Chinese population, 384 subjects with ischemic stroke and 425 healthy control subjects were enrolled and three different *AGER* SNPs were examined (82G/S, −429T/C and −374T/A). Only the 82G/S *AGER* SNP (rs2070600) was associated with the risk for ischemic stroke. Of the homozygote carriers (82S/S), there were generally higher levels of inflammation, as evidenced by lower serum sRAGE levels, and higher levels of serum IL6, hsCRP and PAI1 (Cui et al., 2013). In contrast, the SNPs at −374 and −429 loci demonstrated no association with stroke risk or the levels of inflammatory markers. In a distinct study examining risk for hemorrhagic stroke in a Chinese population, it was found that in subjects less than or equal to 50 years of age, the rs1035798 AGER SNP (homozygote) was associated with increased risk of hemorrhagic stroke (Liu et al., 2015). Further, in a Chinese population, *AGER* rs2070600 and *HMGB1* 2249825 were found to bear relationships to stroke (Li et al., 2017b).

Finally, Montaner and colleagues probed whether measurement of RAGE ligand S100B and sRAGE levels (as well as other markers) might aid in differentiating ischemic vs. hemorrhagic stroke. Admission blood samples obtained from 915 patients (776 with ischemic stroke and 139 with hemorrhagic stroke) within 24 h of the event onset revealed that in samples obtained within the first 6 h (or even in the first 3 h after symptoms) of stroke, increased S100B levels and decreased sRAGE levels were found in hemorrhagic vs. ischemic stroke. The authors speculated that measurement of these S100B and sRAGE markers might aid in differentiation of the two forms of stroke (Montaner et al., 2012). Experiments in animal models tested if the RAGE pathway exerted mediating roles in these disorders.

5.1. Ischemic stroke

In a murine model of focal cerebral ischemia, the effects of neuronal RAGE expression on ischemic stroke were tested in transgenic mice expressing either a full-length form of RAGE or a cytoplasmic domain-deleted form of RAGE in neurons. Compared to normal control animals, neuronal RAGE overexpressing mice displayed significantly increased stroke volume while a trend towards decreased stroke volume was observed in the animals with cytoplasmic domain-deleted RAGE, in which RAGE signaling was reduced (Hassid et al., 2009). In a model of global cerebral ischemia induced by bilateral common carotid artery occlusion, wild-type mice, global *Ager* null mice and transgenic mice expressing esRAGE (as decoy receptor) were tested. A time course study in that model was first performed in wild-type mice, which revealed that increased expression of RAGE was first noted in vascular cells within 12 h of injury and later, between 24 h and 7 days after injury, RAGE expression was increased in glial cells in the hippocampus. Compared to the wild-type mice, mice globally devoid of *Ager* or mice expressing esRAGE displayed higher numbers of surviving neurons in the CA1 region of the hippocampus, in parallel with lower degrees of oxidative stress. After 12 h, the expression of inflammatory markers, such as TNFα and iNOS was lower in the *Ager* null and the esRAGE mice vs. the wild-type, consistent with reduced glial inflammation (Kamide et al., 2012).

Others probed the effects of RAGE and its ligand HMGB1 in ischemic stroke in a mouse model. After the authors demonstrated that HMGB1 was elevated in human stroke patients, they reported that HMGB1 was released from ischemic brain tissue in a murine model of cerebral ischemia. Mediating roles for HMGB1 in stroke were demonstrated by reduction of brain damage in mice treated with either antibodies to HMGB1 or an antagonist to this RAGE ligand. As *in vitro* studies suggested that microglia RAGE contributed to the toxic effects of HMGB1, wild-type mice were subjected to lethal irradiation and transplantation with either *Ager* null or wild-type bone marrow. Those studies revealed that recipients of *Ager* null bone marrow demonstrated reduced infarct size compared with those mice receiving wild-type bone marrow and subjected to ischemic stroke (Muhammad et al., 2008). In distinct studies, it was shown that in murine ischemic stroke, RAGE drives a pro-inflammatory and blunting of anti-inflammatory polarization in diabetic mice (Khan et al., 2016). Interestingly, type 1 diabetic rats treated with Niaspan, a cholesterol-lowering agent, during transient middle cerebral artery occlusion, displayed diminished HMGB1-RAGE-dependent inflammatory profiles (Ye et al., 2011). Given that RAGE has previously been implicated in regulating the cholesterol efflux protein, ABCG1 (Daffu et al., 2015), those data suggest that RAGE may drive inflammatory dysregulation of CNS cells during stroke, at least in part, through impairment of cholesterol homeostasis. More work, however, is required to elucidate the precise mechanisms and connections. Collectively, these studies in ischemic stroke models suggest roles for RAGE in exacerbating the pathogenesis of stroke damage and implicate RAGE actions in vascular, immune/inflammatory and neuronal cells, perhaps in a time-dependent manner.

5.2. Intracerebral hemorrhage (ICH)

An antagonist of RAGE, FPS-ZM1, which blocks binding of ligands such as HMGB1 and S100B to the V-type Ig extracellular domain of RAGE, was employed in a rat and murine model of ICH. In a rat model of ICH, induced by collagenase, it was shown that release of HMGB1 results in expression of VEGF, thereby increasing pathological angiogenesis. In that model, a time-dependent increase in expression of RAGE and HMGB1 was noted in the ipsilateral striatum after induction and up to 14 days later. Treatment with FPS-ZM1 suppressed expression of VEGF and vessel density after induction of hemorrhage in this model (Yang et al., 2015). In a distinct species (mice) and with the use of autologous arterial blood injection into the basal ganglia, ICH was induced in wild-type mice. By 12 h after the injury, expression of RAGE and HMGB1 was increased, in parallel with increased expression of NF-κB p65 and increased permeability of the BBB, brain edema, motor dysfunction and neve fiber injury. Paralleling these markers of damage, local levels of IL1β, IL6, IL8R, and MMP9 were also elevated. In mice treated with FPS-ZM1, however, all of these pathological markers were reduced compared to vehicle-treated mice undergoing ICH (Lei et al., 2015). Together, these findings highlight the importance of ligand-RAGE signaling in the pathophysiology of ICH.

5.3. Subarachnoid hemorrhage (SAH)

In contrast to ICH, SAH is characterized by bleeding between the brain and the skull. Once blood accumulates in that region within the CSF, it causes inflammation in the surrounding brain tissue and increases pressure onto the brain as a result of edema. In model systems, such as the rat, SAH may be induced by injection of autologous blood into the prechiasmatic cistern. Induction of hemorrhage by this method in rats was shown to be associated with increased inflammation, as evidenced by increased expression of RAGE ligands HMGB1 and S100 proteins. In this rat model, expression of RAGE and nuclear NF-κB p65 was significantly increased, particularly in neurons and in microglia, but not in astrocytes (Li et al., 2014). The effects of the RAGE antagonist FPS-ZM1 were tested in this model. These studies revealed that at one day after SAH, the mice treated with the RAGE antagonist

displayed reduced brain edema and better neurological score vs. vehicle. However, by 3 days after hemorrhage, the RAGE antagonist-treated mice displayed increased neuronal cell death with higher levels of apoptosis and diminished autophagy (Li et al., 2017a). These data suggested that, at least in mice and in this model system, RAGE may play both damaging and protective roles, perhaps based on its actions in distinct cell types and on the timing of the post-SAH response. Although the work in that study did not provide insight into potentially contrary cell type-specific roles for RAGE, it is nevertheless important to note that neurons and microglia prominently expressed RAGE after the induction of the SAH.

Finally, in distinct work in the rat model of SAH, the specific effects of RAGE ligand HMGB1 were addressed. In that study, the authors found that the highest expression of HMGB1 occurred on day 14 after the hemorrhage. The rats undergoing SAH were treated with two different inhibitors of HMGB1 secretion (ethyl pyruvate or glycyrrhizin) or the RAGE antagonist, FPS-ZM1. These treatments resulted in reduced expression of growth factors and reduced proliferation of cortical neurons. The authors hypothesized that the redox status form of the HMGB1 might regulate its damaging vs. protective effects. Indeed, compared to administration of recombinant HMGB1, administration of oxidized HMGB1 failed to stimulate pro-inflammatory cytokine production and exhibited protection in the brain, as neurotrophin expression was increased, in parallel with brain recovery (Tian et al., 2017). The authors speculated that in late stages after SAH, HMGB1 may be protective, particularly in the oxidized form.

In summary, RAGE and its ligands appear to contribute to the response to ischemic stroke and to stroke induced by various forms of hemorrhage (intracerebral vs. subarachnoid). The finding that the administration of inhibitors to RAGE ligands or to RAGE itself is not uniformly beneficial or deleterious underscores the possibility that RAGE plays differential roles depending on whether the cells are vascular cells (EC and SMC), immune/inflammatory cells such as microglia, astrocytes and infiltrating immune cells, or neurons. Further, the effects of RAGE actions may be protective and/or deleterious, perhaps depending on the timing in the post-stroke or post-hemorrhage period. In order to provide support for RAGE as a potential target for therapeutic intervention in these disorders, it will be necessary to test these concepts using cell type- and time-dependent deletion of *Ager*, which can be achieved, for example, by using the cre-lox recombinase technique. Further, more extensive time course studies with RAGE or ligand inhibitors are essential, that is, varying the time post-stroke event at which the inhibitors are begun/terminated. Also, further elucidation of the concentrations of these compounds that may be detrimental vs. beneficial in stroke subjects will be important to delineate. Only by such meticulous approaches may the full roles for RAGE and its ligands in these disorders be uncovered and the potential for targeting this axis in stroke and hemorrhage of the CNS be potentially realized.

In the sections to follow in this review, the role of the RAGE signaling axis in disorders of neurodegeneration will be discussed.

6. The RAGE axis and neurodegeneration

The RAGE axis has been implicated in a number of neurodegenerative disorders (Derk et al., 2018b). In this review, the influence of this pathway on AD, amyotrophic lateral sclerosis (ALS) and Parkinson's Disease will be considered. Of note, however, although not covered in this review, work has been published suggesting links of RAGE to Huntington's disease and Creutzfeldt-Jakob disease (Anzilotti et al., 2012; Sasaki et al., 2002).

6.1. Alzheimer's disease

RAGE is expressed in multiple cell types germane to the pathogenesis of AD, including neurons, microglia, astrocytes and ECs; in recent work, the expression of the RAGE cytoplasmic domain binding partner, DIAPH1 has been assessed in AD as well. In that work, the medial temporal cortices of AD patients and aged-matched controls were tested for DIAPH1 expression patterns in multiple cell types, including endothelial cells, oligodendrocytes, neurons, astrocytes, pericytes and myeloid cells. In the case of endothelial cells, the expression of the endothelial marker, Claudin-5, was significantly higher in the AD brain compared to the non-demented brain, but there was no change in relative DIAPH1 intensity within endothelial cells. In oligodendrocytes, marked by expression of myelin basic protein (MBP), no changes were observed for DIAPH1 and total MBP expression within non-demented aging and AD brains. In the case of the marker for neurons, microtubule-associated protein 2 (MAP2), a relative decrease of MAP2 expression in the AD brain was noted compared to the non-demented brain. However, there was no associated change in DIAPH1 intensity. In the case of astrocytes, although an increase in GFAP-positive area was observed in the AD brain, as the area of GFAP positivity increased, so did DIAPH1-positive/GFAP-positive overlap area. Thus, there was no specific change in astrocyte DIAPH1 noted in AD versus the non-demented control brain. Testing for pericyte colocalization with DIAPH1 through the use of an α-Smooth Muscle Actin (α-SMA) antibody revealed that there was no colocalization in either the non-demented or AD brain.

In contrast to the above cell types, however, in the case of myeloid cells, using CD68 as a marker, a significant increase in overlap area between DIAPH1 and CD68 and a significant increase in DIAPH1 intensity within $CD68^{+}$ cells in the AD brain relative to the non-demented control brain was observed. Thus, unlike the other cell types noted above, DIAPH1 expression patterns within myeloid cells were found to be significantly increased in AD. Notably, DIAPH1 expression in myeloid cells correlated with increased lipid staining and inflammatory morphology (Derk et al., 2018a).

Genome wide association studies (GWAS) implicated aging (Chauhan et al., 2015) and inflammatory pathways and activation of microglia in the pathogenesis of AD (Guerreiro et al., 2013; Jonsson et al., 2013; Villegas-Llerena et al., 2016; Zhang et al., 2015). Hence, given the roles for RAGE in inflammatory mechanisms, potential genetic links between *AGER* and AD were studied. As *AGER* is one of the genes in the Human Leukocyte Antigen (HLA) Class III region of the Major Histocompatibility Complex (MHC), the potential implications of *AGER* SNPs with AD were studied in 194 Italian patients with AD and 454 healthy controls. The −374 and −429 AGER SNPs were studied, along with those for *TNFA* and the results of haplotype reconstruction studies suggested that the HLA Class III region might be implicated in AD susceptibility (Maggioli et al., 2013), which was affirmed in a follow-up study in which *HSP70* SNPs were also considered (Boiocchi et al., 2015). In another study of the Alzheimer's Disease Neuroimaging Initiative (ADNI), *AGER* rs2070600 (G82S) was found to be associated with the atrophy rate of the right hippocampus CA1 over two years (Wang et al., 2017c). In a Japanese population, 4 AGER SNPs were studied (rs1800624, rs1800625, rs184003 and rs2070600) in 288 subjects with AD, 76 with Lewy body dementia and 105 age-matched controls. In that study, *AGER* rs184003 was associated with an increased risk of AD and haplotype analyses detected genetic associations between AD and the *AGER* gene (Takeshita et al., 2017). In a European cohort, the *AGER* SNP G82S (rs2070600) was associated with an increased risk of AD in 316 AD patients and 579 controls, but there was no interaction between this *AGER* SNP and the *APOE4* or with mini-mental examination scores (Daborg et al., 2010).

On account of the demonstrated association between diabetes and AD and mild cognitive impairment (MCI) (Cukierman et al., 2005; Huang et al., 2014; Ruiz et al., 2016; Xu et al., 2004), the *AGER* G82S SNP (rs2070600) and levels of AGEs and soluble RAGE were examined in 167 hospitalized type 2 diabetic subjects, of whom 82 were diagnosed with MCI and the other 85 were considered non-MCI controls. Patients with MCI demonstrated significantly lower levels of sRAGE and higher levels of serum AGE-peptide compared to control subjects. In

that study, the *AGER* SNP G82S bore no relationship to MCI in the type 2 diabetic subjects (Wang et al., 2016). Collectively, these data suggest that at least in certain populations, RAGE may affect genetic risk for AD or MCI and that in diabetes, the levels of the decoy receptor, sRAGE, are lower in individuals with cognitive dysfunction. Hence, components of the RAGE pathway may hold promise as biomarkers and/or predictors of AD and MCI. Experimental models have probed the cell type specific effects of RAGE using murine models of pathology.

Roles for RAGE in neurons in animal models of AD were directly tested in the commonly used transgenic mouse models of overexpression of mutant amyloid precursor protein (APP) in combination with either full-length or cytoplasmic domain-deleted *Ager* selectively in neurons. Overexpression of neuronal *Ager* accelerated behavioral abnormalities and altered activation of markers of synaptic plasticity and neuropathological abnormalities; these pathologies were observed at time points at which the mutant APP mouse control had yet to exhibit any pathologies. In contrast, in the mutant APP mice expressing the cytoplasmic domain-deleted *Ager*, and thus with suppressed ligand-RAGE signaling, attenuation of behavioral, and neuropathological changes was observed compared to the control mutant APP mice (Arancio et al., 2004). Additional studies suggested that one of the mechanisms by which neuronal RAGE expression activated distinct cells in the AD brain and in AD-like mouse models was through neuronal expression of macrophage colony stimulating factor (MCSF), which consequently triggered activation of microglia (Du Yan et al., 1997).

Other studies focused on expression of RAGE in microglia in neuronal-specific mutant APP mice. In transgenic mutant APP mice expressing either full-length *Ager* or cytoplasmic domain-deleted *Ager* in myeloid cells, whereas overexpression of RAGE increased neuroinflammation (IL1β and TNFα), microgliosis and astrocytosis, accumulation of Aβ and behavioral abnormalities, deletion of the RAGE cytoplasmic domain in microglia in this model exerted protection against these abnormalities compared to those observed in the mutant APP mice (Fang et al., 2010). Of note, the promoter used to drive the expression of RAGE and cytoplasmic domain-deleted RAGE forms in these animals also impacted expression in peripheral myeloid cells and through the development of the organism, which may alter the profile of these cells irrespective of the mutant APP background. Hence, the findings observed in these transgenic mice cannot be solely attributed to the effects of RAGE in adult and aged microglia. In distinct experiments, others tested microglia (myeloid)-cytoplasmic domain-deleted *Ager* in entorhinal cortex dysfunction in mutant APP mice. Early abnormalities in long term potentiation (LTP) in the entorhinal cortex and in associative behavior tasks *in vivo* were also observed in these mice. Evidence of activated stress related kinases (p38 MAPK and JNK pathway) was also attenuated in the transgenic vs. mutant APP alone mice (Criscuolo et al., 2017). These studies specifically examined a highly vulnerable region of the brain early in the course of neuronal deficits in an AD-like mouse model and implicated roles for myeloid/microglia RAGE in the pathogenesis of AD.

In cultured BV2 microglia-like cells, the effects of RAGE ligand AGEs on signal transduction were probed. AGEs activated Rho-associated protein kinase (ROCK) in a manner suppressed by the ROCK inhibitor fasudil or by the RAGE inhibitor FPS-ZM1. AGE-mediated upregulation of ROS, iNOS, COX2, NLRP3 and nuclear NF-κB p65 was attenuated in the BV2 cells by fasudil or FPS-ZM1. AGEs increased expression of pro-inflammatory "M1"-like markers in BV2 cells and decreased expression of anti-inflammatory "M2"-like markers, in a RAGE- and ROCK-dependent manner (Chen et al., 2017). Although "M1" and "M2" markers of the state of inflammation reflect solely *in vitro* designations, these experiments nevertheless suggested that RAGE ligands might affect pro/anti-inflammatory gene programs in BV2 cells. Further, the extent to which BV2 cells truly model *in vivo* microglia must also be considered in interpreting such studies.

In addition to neurons and microglia, RAGE is also prominently expressed in ECs and studies using AD-like mouse models demonstrated the prominent role of RAGE in transporting Aβ across the BBB and via upregulation of inflammatory mediators and endothelin-1 (ET1), RAGE contributed to Aβ-mediated vasoconstriction and suppression of cerebral blood flow (CBF). In mutant APP transgenic mice, administration of sRAGE blocked the adverse effects of the Aβ-RAGE interaction (Deane et al., 2003). In other studies, RAGE was shown to downregulate expression of low density lipoprotein receptor-related protein 1 (LRP1) (Deane et al., 2004). As LRP1 is responsible for Aβ clearance from the brain, such findings strongly suggest that RAGE plays key roles in overall Aβ transport and load in the brain, at least in part through perturbation of the BBB, CBF and expression of LRP1.

In other studies, the effect of hypertension, which is associated with AD, was assessed on the brain vasculature in the context of Aβ. In C57BL/6 mice, hypertension was induced by transverse aortic coarctation (TAC); in that model, by four weeks post-procedure, cerebral amyloid deposition is noted. In parallel, immunohistochemistry studies noted an early (within hours of TAC) and sustained upregulation of RAGE in brain blood vessels in the cortex and hippocampus after TAC. Impaired learning and memory was also observed in the TAC-treated animals. However, in mice globally devoid of *Ager,* evidence of significant protection was noted as follows: 1) reduced cerebral amyloid deposition; 2) improved performance in behavior studies; and 3) reduced oxidative stress; these effects were recapitulated by treatment of wild-type mice undergoing TAC and treated with FPS-ZM1 (Carnevale et al., 2012). Interestingly, in that work it was shown that induction of TAC increases levels of another RAGE ligand, AGEs. Collectively, these studies suggest that in hypertension, multiple RAGE ligands may be generated, which, at least in part via RAGE, aggravate pro-inflammatory and pro-oxidative pathways leading to cellular stress and organ damage.

The effects of RAGE on the BBB have also been addressed in *in vitro* models. For example, in a monolayer BBB model composed of murine ECs (bEnd.3 cells), Aβ(1–42) was shown to increase "BBB leakage" and result in reduction of tight junction scaffold proteins ZO1, claudin-5 and occludin. Incubation of these cells with Aβ(1–42) significantly upregulated their expression of RAGE; when these cells were treated with anti-RAGE IgG or when siRNAs were employed to reduce *Ager* expression, the effects of Aβ(1–42) on expression of scaffold proteins was blocked as well as the upregulation of MMP2 or MMP9. Further, an inhibitor of MMPs (GM6001) also prevented the detrimental effects of Aβ(1–42) on BBB leakage and on expression of tight junction proteins (Wan et al., 2015). In other studies performed in bEnd.3 cells, treatment with Aβ(1–42) increased permeability, disrupted ZO1 expression and increased secretion of intracellular calcium and MMPs, which was prevented by anti-RAGE antibodies (Kook et al., 2012).

6.2. Amyotrophic lateral sclerosis (ALS)

ALS is a complex disorder in which the primary target for dysfunction and death is the motor neuron. Multiple hypotheses have been put forth with respect to the cause of ALS, such as but not limited to increased toxicity from glutamate, increased oxidative stress (increased ROS and reactive nitrogen species (NOS)), protein aggregation and disruptions in proteostasis, defects in RNA processing, endoplasmic reticulum and mitochondrial stress, dysfunction of axonal transport mechanisms, and environmental factors, such as toxins or certain classes of infections (Lyon et al., 2019). Yet, multiple cell types, such as microglia, peripheral monocytes/macrophages, astrocytes and T and B lymphocytes have been postulated to play contributing roles to ALS pathology, especially to its progression and motor neuron death, contributing to the overall presumption that ALS is not a cell-autonomous disease. In this context, RAGE is expressed on many of these cell types and has been studied in both human and animal models of ALS.

In human subject spinal cord, compared to healthy control subjects, ALS spinal cord demonstrated higher levels of RAGE and its ligands,

particularly carboxy methyllysine (CML)-AGE, S100B and HMGB1 (Juranek et al., 2015). Other studies addressed expression patterns of RAGE as well as toll-like receptors 2 and 4 (TLR2 and TLR4) and ligand HMGB1 in 12 sporadic ALS and 6 control subjects. In ALS subjects, TLR2, TLR4 and RAGE were found to be highly expressed in reactive glial cells in the ventral horn and white matter; TLR2 was shown to be predominantly expressed in microglia, whereas RAGE and TLR4 were strongly expressed in astrocytes. By real-time qPCR, levels of HMGB1 mRNA were also shown to be increased in ALS vs. control spinal cord and the protein signal was specifically noted in the cytoplasm of reactive glial cells (Casula et al., 2011). Others examined patterns of sRAGE levels in 20 ALS patients and in 20 control subjects and reported that sRAGE levels were significantly lower in serum of the ALS vs. control individuals and that there was no correlation between the levels of serum sRAGE and disease clinical parameters in the ALS patients; however, it is notable that only 20 ALS patients were examined in the study and whether the work was powered to address such correlations is not clear (Ilzecka, 2009).

Experiments in cultured cellular models for ALS have also assessed a potential role for RAGE in this disease. Prompted by the observation that post-translationally modified forms of nerve growth factor (NGF) were observed in the spinal cord of murine ALS models, these concepts were tested in cellular models. *In vitro* glycation of NGF promoted its oligomerization and resulted in the generation of modified NGF as a RAGE ligand, which induced motor neuron death in culture and astrocyte-mediated motor neuron toxicity and similar findings were observed for nitrated NGF (Kim et al., 2018). In NSC-34 motor neuron like cells, transfection with mutant SOD1 resulted in production of exosomes by those cells, which led to increased expression of microRNA (miRNA) 124 and HMGB1 mRNA and protein. When exosomes from mutant SOD1-transfected NSC-34 cells vs. control were incubated with N9 microglia-like cells, highly significant upregulation of IL10, Arginase 1, TREM2, RAGE and TLR4 was noted. Further, increased expression of HMGB1, miR124, miR146a and miR155 and an increase in inflammatory markers, such as activated NF-κB, was observed as well (Pinto et al., 2017). Strikingly, in N9 cells exposed to the mutant SOD1-transfected NSC-34 cell exosomes, a loss of phagocytic ability and induction of senescence (as noted by senescence-associated β-galactosidase staining) was noted (Pinto et al., 2017). Although direct roles for RAGE in these experiments were not tested, its upregulation in the cells treated with the mutant SOD1-related exosomes suggest that one of the responses of microglia to these apparently toxic species is upregulation of receptors such as RAGE, in parallel with its ligands.

In addition, roles for S100B in ALS were considered, particularly as S100B is released from astrocytes. In a rat model of ALS, *SOD1*^{G93A} modified rats demonstrated a time dependent significant increase in S100B expression in spinal cord astrocytes, which colocalized with increased expression of RAGE. In primary astrocytes from mouse pup cortices, siRNA-knockdown of S100B resulted in a suppression of genes known to be upregulated in ALS, including GFAP, TNFα, CXCL10, and CCL6 (Serrano et al., 2017).

In other studies, potential neuroprotective roles for HMGB1 were tested. Spinal motor neurons from wild-type and *SOD1*^{G93A} mice displayed differential intracellular expression patterns of HMGB1; an increase in HMGB1 shuttling from the nucleus to the cytoplasm of these cells was observed in the mutant vs. wild-type mice, suggesting, perhaps, that HMGB1 in the *SOD1*^{G93A} mice might play protective vs. deleterious roles. Only the astrocytes from wild-type but not the *SOD1*^{G93A} mice were able to increase BDNF and GDNF levels upon stimulation with HMGB1, in a RAGE-dependent manner, suggesting that a beneficial role for astrocyte HMGB1 in neuronal growth factor expression may be dysregulated in ALS (Brambilla et al., 2018).

In vivo studies, to date, testing the role of RAGE in murine models of ALS are limited. In one study, sRAGE was administered to male *SOD1*^{G93A} mutant mice (B6/SJL background) beginning at age 8 weeks and continued until sacrifice. Compared to vehicle-treated animals, life span was extended and progression of ALS symptomatology was delayed by sRAGE treatment. At sacrifice, in the spinal cord tissue, sRAGE-treated animals displayed significantly higher numbers of neurons and lower number of astrocytes vs. the vehicle-treated group (Juranek et al., 2016). In that study, however, the mechanisms by which sRAGE exerted its benefit were not addressed, nor were distinct time courses for initiation of sRAGE administration tested.

Indeed, other studies, in an unrelated model of spinal cord injury (SCI), complex roles for RAGE in repair were uncovered in mice and rats. In a murine model of SCI, it was shown that RAGE expression rose within 12 h after the injury in WT mice; in *Ager* null mice subjected to the same degree of SCI, repair was improved, as evidence by improved functional outcomes, reduced expression of GFAP, and reduced inflammation, as indicated by lower levels of IL1β, TNFα, IL6, and NF-κB activation (Guo et al., 2014). Yet, in a rat model of SCI, animals undergoing the injury received an injection of anti-RAGE antibody directly into the central site of the injury. In contrast to induction of pro-repair mechanisms, the anti-RAGE antibody treatment resulted in reduced neuronal survival, disruption of Wnt/β-catenin signaling, and higher levels of autophagy (Mei et al., 2019; Wang et al., 2017a, 2018). Of course, the species under study are different and the mode of RAGE antagonism was dramatically different, that is, global deletion of *Ager* through development to adulthood in mice or treatment of adult rats with an antibody to RAGE. In any case, however, these studies underscore the pleiotropic roles that RAGE appears to play in neuronal system repair in the spinal cord.

Given the temporal, cell-type and complexity of cell intrinsic vs. cell-cell communications in the pathobiology of ALS and SCI, these studies raise many important questions. For example, it is not clear if sRAGE crosses the BBB. If the principal site of action of this agent was in the periphery, such as on the phenotype of peripheral monocytes and/or on activities at the neuromuscular junction (NMJ), remains an open question that can certainly be addressed experimentally. Also, in light of its close link to RAGE, future studies must examine if there are roles for DIAPH1 in the ALS. In addition, potential roles for breakdown of the blood-spinal cord barrier and roles for vascular cells in ALS, such as in the context of RAGE, require further investigation.

6.3. Parkinson's disease

Increasing evidence links RAGE to Parkinson's disease in human subjects and in animal models (Jiang et al., 2018). For example, pathological analyses were performed in human subject brains of asymptomatic Parkinson's disease with incidental Lewy body disease-related changes versus healthy age-matched controls. Increased expression of RAGE ligand AGEs was identified in the substantia nigra, amygdala, and frontal cortex. In parallel, increased expression of RAGE was noted in the substantia nigra and frontal cortex in human subject cases with early stages of parkinsonian neuropathology (Dalfo et al., 2005). Furthermore, in human subjects, *AGER* gene polymorphisms were studied in 285 Parkinson's Disease patients versus 285 healthy control subjects in the Chinese Han population. The only *AGER* SNP that showed a significant difference between the Parkinson's Disease patients and the controls was the 429T/C polymorphism. The carriers of the −429C allele exhibited a decreased risk of Parkinson's Disease, thereby suggesting that the −429T/C SNP may be a protective factor for Parkinson's Disease, at least in the Chinese Han population (Gao et al., 2014).

Studies in animal models have also begun to probe the role of the RAGE axis in the pathogenesis of Parkinson's Disease-like pathologies. Examples of some of these findings in animals with Parkinson's Disease-like pathologies are as follows: In the classical model for Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), deletion of *Ager* afforded protection in nigral dopaminergic neurons from cell death. Further, as NF-κB has been implicated in the pathogenesis of neuronal injury in this disease, the activation of this

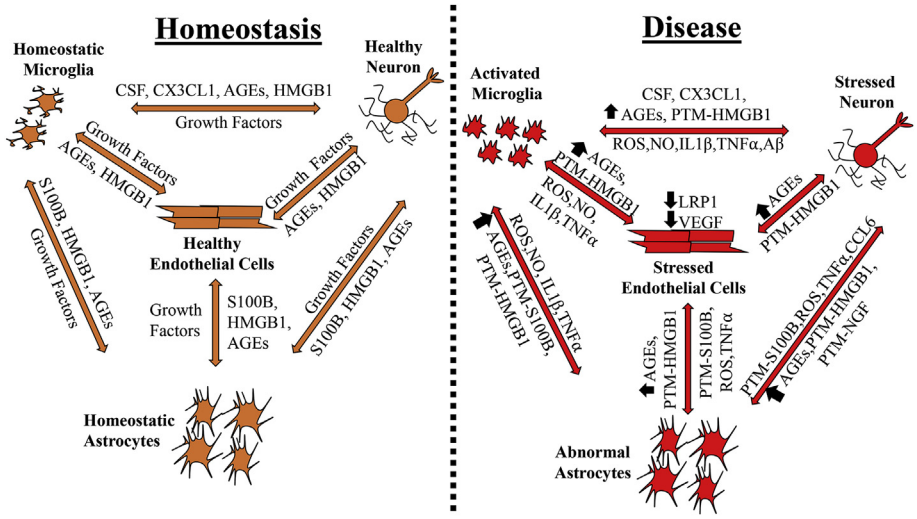

Fig. 3. Working model of RAGE-dependent cross-talk among microglia, neurons, astrocytes and endothelial cells in homeostasis and disease. In homeostasis, these cell types, and others not shown, secrete a number of protective factors, such as growth factors, physiological RAGE ligands, and chemokines. Importantly, blocking physiological RAGE ligands can disrupt homeostasis, as these factors have beneficial functions. In disease, reduced growth factor secretion, such as of BDNF and NGF, may suppress adaptive stress responses and prevent repair. Further, the release of higher concentrations of RAGE ligands (and, perhaps, their oligomerization) and the post-translationally modified (PTM) forms of these ligands from stressed cells may promote pathological RAGE signal transduction. These stressed cells release reactive oxygen species (ROS) and nitric oxide (NO), thereby further promoting wide-spread cellular stress. We posit that targeting pathological RAGE-dependent signaling by proper timing of therapeutic intervention will be key to reducing cellular stress in the CNS, without impacting the homeostatic RAGE-dependent signaling.

factor was tested in *Ager* null and *Ager* expressing mice after MPTP treatment. In the absence of *Ager,* the nuclear translocation of the NF-κB subunit p65 in dopaminergic neurons and glial cells was significantly inhibited, thereby suggesting roles for RAGE in activation of this pro-injury factor. Consistent with this premise, the increased apoptotic cell death induced by MPTP was attenuated in mice devoid of *Ager* (Teismann et al., 2012).

In other work, Gasparotto and colleagues reported that rats treated with 6-hydroxydopamine (6-OHDA) demonstrated increased expression of RAGE in the substantia nigra, in parallel with increased activation of NF-κB, astrocytosis and microgliosis. In rats treated with 6-OHDA and the RAGE inhibitor FPS-ZM1, these adverse effects were reduced and the locomotion and exploratory deficits in the rats induced by 6-OHDA were also attenuated (Gasparotto et al., 2017).

Recently, umbilical cord blood-derived mesenchymal stem cells were CRISPR/Cas9-edited to secrete sRAGE on the premise that local sequestration of RAGE ligands, such as AGEs, might mitigate Parkinson's like pathology. These sRAGE-edited cells were then injected into the corpus striatum of rotenone-treated mice, a Parkinson's Disease-like model. It was reported that neuronal death in the corpus striatum and substantial nigra was significantly reduced in the presence of the sRAGE-expressing injected cells, in parallel with reduced movement abnormalities in the rotenone-treated animals (Lee et al., 2019).

Collectively, these examples of in *vivo* studies targeting the RAGE axis in models of Parkinson's Disease link this receptor and its ligands to this disease. This work therefore suggests that testing RAGE antagonism in subjects with Parkinson's Disease may be logical and timely. In the section to follow, a novel strategy to target the RAGE axis will be considered.

7. Targeting RAGE signal transduction – A novel therapeutic approach

A small molecule antagonist of RAGE, Azeliragon, which targets the binding of RAGE ligands to the extracellular domains of RAGE, failed to show benefit compared to placebo in human subjects with mild AD (VTV Therapeutics, 2018). Multiple experiments have shown that the extracellular domains of RAGE, particularly the V-type Ig domain, are heterogeneous with respect to ligand binding; ligands may bind to distinct pockets on the V-domain such as in hydrophobic or charged sites and certain ligands may preferentially bind at the C-type Ig domains (Koch et al., 2010; Kumano-Kuramochi et al., 2009; Leclerc et al., 2009; Park et al., 2010; Xie et al., 2007, 2008). Such data suggest that targeting the extracellular domains of RAGE might not be an effective strategy to suppress RAGE activity in these disorders, especially given that there is no evidence that individual RAGE-related diseases may be influenced by only one or a very small number of ligands. Rather, multiple cellular stress-related RAGE ligands appear to populate diseased tissues, such as AGEs and S100 molecules in human diabetic atherosclerosis (Burke et al., 2004).

In contrast, strategies that target the intracellular domain of RAGE may be effective, given that the cytoplasmic domain of RAGE is small (less than 45 amino acids) and is essential for RAGE ligand-mediated cellular signaling. As discussed above, the discovery of DIAPH1 as a putative effector of RAGE signaling has unveiled a potentially more strategic therapeutic target. Early work has identified small molecules that block the binding of the RAGE cytoplasmic domain to DIAPH1 and have shown efficacy against the effects of RAGE ligands on signal transduction and changes in gene and functional expression endpoints in *in vitro* and *in vivo* studies (Manigrasso et al., 2016).

If and how such a strategy may be beneficial in RAGE/DIAPH1-related neurovascular and neurodegenerative disorders remains to be tested.

8. Perspectives and future directions

Studies in human subjects and animal models are steadily linking RAGE to the vulnerability to and pathogenesis of neurovascular and neurodegenerative disorders, particularly with respect to driving increased inflammation and barrier dysfunction coincident with aging. No doubt the biology of RAGE is complex and much needs to be learned about the homeostatic/pathobiological roles of this receptor and its downstream effector, DIAPH1. Insights into RAGE's innate functions have emerged from the observations that stress-related inflammation accompanies acute environmental and metabolic cues that shape host defenses. Recent work in primates and rodents has suggested that conserved amino acid residues on the extracellular ligand- and intracellular adaptor-binding regions and receptor oligomerization-related surfaces might convey “adaptive fitness,” that is, such sites on RAGE might confer advantages for host defense and rapid responses to challenge (Wu et al., 2015). Hence, in neurovascular and neurodegenerative disorders, it is plausible that RAGE and its ligands play both beneficial/adaptive and deleterious roles (Fig. 3).

How may such complexities be rectified? Experiments using transgenic mice to imbue tissue-specific and temporally-regulated modulation of RAGE (and DIAPH1) expression, as well as inhibitors of RAGE/

DIAPH1 that may be administered at early onset, mid-progressing phase and at late, end-stage of disease, will be essential to dissect the innate vs. deleterious roles of RAGE and DIAPH1 and to identify the optimal conditions for therapeutic interruption of RAGE signaling. Further, as some studies suggest that post-translational modifications of key RAGE ligands in the CNS, such as HMGB1, may dictate their adaptive vs. deleterious functions, consideration of the physical state of the RAGE ligands will be important. Finally, as a mountain of evidence suggests that RAGE-dependent physiologic vs. pathologic roles in the CNS may be both cell intrinsic and/or dependent on cell-cell networking and communication in vascular and neurodegenerative disorders, experiments testing such concepts *in vitro* and *in vivo* utilizing methods that consider RAGE-dependent cell-cell communication in mixed cultures and, possibly, organoid models (Wang et al., 2017b), will be essential. In this context, complimentary experiments using transcriptomic, proteomic, and metabolomic approaches may aid in uncovering how RAGE and DIAPH1 respond to toxic and detrimental cues in the CNS.

As there is mounting experimental evidence that there are scenarios in which blocking RAGE exerts benefit in the CNS pathology, identifying and harnessing the precise conditions for effectively targeting RAGE will be essential to treat chronic disorders of the CNS, especially those that accompany aging, cerebral ischemia, and diabetes.

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References

Aleshin, A., Ananthakrishnan, R., Li, Q., Rosario, R., Lu, Y., Qu, W., Song, F., Bakr, S., Szabolcs, M., D'Agati, V., Liu, R., Homma, S., Schmidt, A.M., Yan, S.F., Ramasamy, R., 2008. RAGE modulates myocardial injury consequent to LAD infarction via impact on JNK and STAT signaling in a murine model. Am. J. Physiol. Heart Circ. Physiol. 294, H1823–H1832.

Anzilotti, S., Giampa, C., Laurenti, D., Perrone, L., Bernardi, G., Melone, M.A., Fusco, F.R., 2012. Immunohistochemical localization of receptor for advanced glycation end (RAGE) products in the R6/2 mouse model of Huntington's disease. Brain Res. Bull. 87, 350–358.

Arancio, O., Zhang, H.P., Chen, X., Lin, C., Trinchese, F., Puzzo, D., Liu, S., Hegde, A., Yan, S.F., Stern, A., Luddy, J.S., Lue, L.F., Walker, D.G., Roher, A., Buttini, M., Mucke, L., Li, W., Schmidt, A.M., Kindy, M., Hyslop, P.A., Stern, D.M., Du Yan, S.S., 2004. RAGE potentiates Abeta-induced perturbation of neuronal function in transgenic mice. EMBO J. 23, 4096–4105.

Avalos, A.M., Kiefer, K., Tian, J., Christensen, S., Shlomchik, M., Coyle, A.J., Marshak-Rothstein, A., 2010. RAGE-independent autoreactive B cell activation in response to chromatin and HMGB1/DNA immune complexes. Autoimmunity 43, 103–110.

Boiocchi, C., Maggioli, E., Monti, M.C., Zorzetto, M., Sinforiani, E., Cereda, C., Ricevuti, G., Cuccia, M., 2015. The possible involvement of HLA class III haplotype (RAGE, HSP70 and TNF genes) in alzheimer's disease. Curr. Alzheimer Res. 12, 997–1005.

Brambilla, L., Martorana, F., Guidotti, G., Rossi, D., 2018. Dysregulation of astrocytic HMGB1 signaling in amyotrophic lateral sclerosis. Front. Neurosci. 12, 622.

Bucciarelli, L.G., Ananthakrishnan, R., Hwang, Y.C., Kaneko, M., Song, F., Sell, D.R., Strauch, C., Monnier, V.M., Yan, S.F., Schmidt, A.M., Ramasamy, R., 2008. RAGE and modulation of ischemic injury in the diabetic myocardium. Diabetes 57, 1941–1951.

Bucciarelli, L.G., Kaneko, M., Ananthakrishnan, R., Harja, E., Lee, L.K., Hwang, Y.C., Lerner, S., Bakr, S., Li, Q., Lu, Y., Song, F., Qu, W., Gomez, T., Zou, Y.S., Yan, S.F., Schmidt, A.M., Ramasamy, R., 2006. Receptor for advanced-glycation end products: key modulator of myocardial ischemic injury. Circulation 113, 1226–1234.

Burke, A.P., Kolodgie, F.D., Zieske, A., Fowler, D.R., Weber, D.K., Varghese, P.J., Farb, A., Virmani, R., 2004. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. Arterioscler. Thromb. Vasc. Biol. 24, 1266–1271.

Carnevale, D., Mascio, G., D'Andrea, I., Fardella, V., Bell, R.D., Branchi, I., Pallante, F., Zlokovic, B., Yan, S.S., Lembo, G., 2012. Hypertension induces brain beta-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension 60, 188–197.

Casula, M., Iyer, A.M., Spliet, W.G., Anink, J.J., Steentjes, K., Sta, M., Troost, D., Aronica, E., 2011. Toll-like receptor signaling in amyotrophic lateral sclerosis spinal cord tissue. Neuroscience 179, 233–243.

Chauhan, G., Adams, H.H.H., Bis, J.C., Weinstein, G., Yu, L., Toglhofer, A.M., Smith, A.V., van der Lee, S.J., Gottesman, R.F., Thomson, R., Wang, J., Yang, Q., Niessen, W.J., Lopez, O.L., Becker, J.T., Phan, T.G., Beare, R.J., Arfanakis, K., Fleischman, D., Vernooij, M.W., Mazoyer, B., Schmidt, H., Srikanth, V., Knopman, D.S., Jack Jr., C.R., Amouyel, P., Hofman, A., DeCarli, C., Tzourio, C., van Duijn, C.M., Bennett, D.A., Schmidt, R., Longstreth Jr., W.T., Mosley, T.H., Fornage, M., Launer, L.J., Seshadri, S., Ikram, M.A., Debette, S., 2015. Association of Alzheimer's disease GWAS loci with MRI markers of brain aging. Neurobiol. Aging 36 1765.e1767-1765.e1716.

Chen, J., Sun, Z., Jin, M., Tu, Y., Wang, S., Yang, X., Chen, Q., Zhang, X., Han, Y., Pi, R., 2017. Inhibition of AGEs/RAGE/Rho/ROCK pathway suppresses non-specific neuroinflammation by regulating BV2 microglial M1/M2 polarization through the NF-kappaB pathway. J. Neuroimmunol. 305, 108–114.

Chesarone, M.A., DuPage, A.G., Goode, B.L., 2010. Unleashing formins to remodel the actin and microtubule cytoskeletons. Nat. Rev. Mol. Cell Biol. 11, 62–74.

Cipollone, F., Iezzi, A., Fazia, M., Zucchelli, M., Pini, B., Cuccurullo, C., De Cesare, D., De Blasis, G., Muraro, R., Bei, R., Chiarelli, F., Schmidt, A.M., Cuccurullo, F., Mezzetti, A., 2003. The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control. Circulation 108, 1070–1077.

Criscuolo, C., Fontebasso, V., Middei, S., Stazi, M., Ammassari-Teule, M., Yan, S.S., Origlia, N., 2017. Entorhinal Cortex dysfunction can be rescued by inhibition of microglial RAGE in an Alzheimer's disease mouse model. Sci. Rep. 7, 42370.

Cui, X., Chen, H., Hou, X., Wang, S., Jayaram, S., Zheng, Z., 2013. Polymorphism of the RAGE affects the serum inflammatory levels and risk of ischemic stroke in a Chinese population. Cell. Physiol. Biochem. 32, 986–996.

Cukierman, T., Gerstein, H.C., Williamson, J.D., 2005. Cognitive decline and dementia in diabetes–systematic overview of prospective observational studies. Diabetologia 48, 2460–2469.

Daborg, J., von Otter, M., Sjolander, A., Nilsson, S., Minthon, L., Gustafson, D.R., Skoog, I., Blennow, K., Zetterberg, H., 2010. Association of the RAGE G82S polymorphism with Alzheimer's disease. J. Neural Transm. 117, 861–867.

Daffu, G., Shen, X., Senatus, L., Thiagarajan, D., Abedini, A., Hurtado Del Pozo, C., Rosario, R., Song, F., Friedman, R.A., Ramasamy, R., Schmidt, A.M., 2015. RAGE suppresses ABCG1-mediated macrophage cholesterol efflux in diabetes. Diabetes 64, 4046–4060.

Dalfo, E., Portero-Otin, M., Ayala, V., Martinez, A., Pamplona, R., Ferrer, I., 2005. Evidence of oxidative stress in the neocortex in incidental Lewy body disease. J. Neuropathol. Exp. Neurol. 64, 816–830.

Deane, R., Du Yan, S., Submamaryan, R.K., LaRue, B., Jovanovic, S., Hogg, E., Welch, D., Manness, L., Lin, C., Yu, J., Zhu, H., Ghiso, J., Frangione, B., Stern, A., Schmidt, A.M., Armstrong, D.L., Arnold, B., Liliensiek, B., Nawroth, P., Hofman, F., Kindy, M., Stern, D., Zlokovic, B., 2003. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat. Med. 9, 907–913.

Deane, R., Wu, Z., Zlokovic, B.V., 2004. RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. Stroke 35, 2628–2631.

Derk, J., Bermudez Hernandez, K., Rodriguez, M., He, M., Koh, H., Abedini, A., Li, H., Fenyo, D., Schmidt, A.M., 2018a. Diaphanous 1 (DIAPH1) is highly expressed in the aged human medial temporal cortex and upregulated in myeloid cells during alzheimer's disease. J. Alzheimer's Dis. 64, 995–1007.

Derk, J., MacLean, M., Juranek, J., Schmidt, A.M., 2018b. The receptor for advanced glycation endproducts (RAGE) and mediation of inflammatory neurodegeneration. J Alzheimers Dis Parkinsonism 8.

Du Yan, S., Zhu, H., Fu, J., Yan, S.F., Roher, A., Tourtellotte, W.W., Rajavashisth, T., Chen, X., Godman, G.C., Stern, D., Schmidt, A.M., 1997. Amyloid-beta peptide-receptor for advanced glycation end product interaction elicits neuronal expression of macrophage-colony stimulating factor: a proinflammatory pathway in Alzheimer disease. Proc. Natl. Acad. Sci. U. S. A. 94, 5296–5301.

Dumitriu, I.E., Baruah, P., Bianchi, M.E., Manfredi, A.A., Rovere-Querini, P., 2005. Requirement of HMGB1 and RAGE for the maturation of human plasmacytoid dendritic cells. Eur. J. Immunol. 35, 2184–2190.

Emanuele, E., D'Angelo, A., Tomaino, C., Binetti, G., Ghidoni, R., Politi, P., Bernardi, L., Maletta, R., Bruni, A.C., Geroldi, D., 2005. Circulating levels of soluble receptor for advanced glycation end products in Alzheimer disease and vascular dementia. Arch. Neurol. 62, 1734–1736.

Fang, F., Lue, L.F., Yan, S., Xu, H., Luddy, J.S., Chen, D., Walker, D.G., Stern, D.M., Yan, S., Schmidt, A.M., Chen, J.X., Yan, S.S., 2010. RAGE-dependent signaling in microglia contributes to neuroinflammation, Abeta accumulation, and impaired learning/memory in a mouse model of Alzheimer's disease. FASEB J. 24, 1043–1055.

Farkas, E., Luiten, P.G., 2001. Cerebral microvascular pathology in aging and Alzheimer's disease. Prog. Neurobiol. 64, 575–611.

Gao, J., Teng, J., Liu, H., Han, X., Chen, B., Xie, A., 2014. Association of RAGE gene polymorphisms with sporadic Parkinson's disease in Chinese Han population. Neurosci. Lett. 559, 158–162.

Gao, X., Zhang, H., Schmidt, A.M., Zhang, C., 2008. AGE/RAGE produces endothelial dysfunction in coronary arterioles in type 2 diabetic mice. Am. J. Physiol. Heart Circ. Physiol. 295, H491–H498.

Gasparotto, J., Ribeiro, C.T., Bortolin, R.C., Somensi, N., Rabelo, T.K., Kunzler, A., Souza, N.C., Pasquali, M.A.B., Moreira, J.C.F., Gelain, D.P., 2017. Targeted inhibition of RAGE in substantia nigra of rats blocks 6-OHDA-induced dopaminergic denervation. Sci. Rep. 7, 8795.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., Cruchaga, C., Sassi, C., Kauwe, J.S., Younkin, S., Hazrati, L., Collinge, J., Pocock, J., Lashley, T., Williams, J., Lambert, J.C., Amouyel, P., Goate, A., Rademakers, R., Morgan, K., Powell, J., St George-Hyslop, P., Singleton, A., Hardy, J., 2013. TREM2 variants in Alzheimer's disease. N. Engl. J. Med. 368, 117–127.

Guo, J.D., Li, L., Shi, Y.M., Wang, H.D., Yuan, Y.L., Shi, X.X., Hou, S.X., 2014. Genetic ablation of receptor for advanced glycation end products promotes functional

recovery in mouse model of spinal cord injury. Mol. Cell. Biochem. 390, 215–223.
Hallam, K.M., Li, Q., Ananthakrishnan, R., Kalea, A., Zou, Y.S., Vedantham, S., Schmidt, A.M., Yan, S.F., Ramasamy, R., 2010. Aldose reductase and AGE-RAGE pathways: central roles in the pathogenesis of vascular dysfunction in aging rats. Aging Cell 9, 776–784.
Hassid, B.G., Nair, M.N., Ducruet, A.F., Otten, M.L., Komotar, R.J., Pinsky, D.J., Schmidt, A.M., Yan, S.F., Connolly, E.S., 2009. Neuronal RAGE expression modulates severity of injury following transient focal cerebral ischemia. J. Clin. Neurosci. 16, 302–306.
Huang, C.C., Chung, C.M., Leu, H.B., Lin, L.Y., Chiu, C.C., Hsu, C.Y., Chiang, C.H., Huang, P.H., Chen, T.J., Lin, S.J., Chen, J.W., Chan, W.L., 2014. Diabetes mellitus and the risk of Alzheimer's disease: a nationwide population-based study. PLoS One 9, e87095.
Hudson, B.I., Kalea, A.Z., Del Mar Arriero, M., Harja, E., Boulanger, E., D'Agati, V., Schmidt, A.M., 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, 34457–34468.
Iadecola, C., 2017. The neurovascular unit coming of age: a journey through neurovascular coupling in Health and disease. Neuron 96, 17–42.
Ilzecka, J., 2009. Serum-soluble receptor for advanced glycation end product levels in patients with amyotrophic lateral sclerosis. Acta Neurol. Scand. 120, 119–122.
Jiang, X., Wang, X., Tuo, M., Ma, J., Xie, A., 2018. RAGE and its emerging role in the pathogenesis of Parkinson's disease. Neurosci. Lett. 672, 65–69.
Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P.V., Snaedal, J., Bjornsson, S., Huttenlocher, J., Levey, A.I., Lah, J.J., Rujescu, D., Hampel, H., Giegling, I., Andreassen, O.A., Engedal, K., Ulstein, I., Djurovic, S., Ibrahim-Verbaas, C., Hofman, A., Ikram, M.A., van Duijn, C.M., Thorsteinsdottir, U., Kong, A., Stefansson, K., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. N. Engl. J. Med. 368, 107–116.
Juranek, J.K., Daffu, G.K., Geddis, M.S., Li, H., Rosario, R., Kaplan, B.J., Kelly, L., Schmidt, A.M., 2016. Soluble RAGE treatment delays progression of amyotrophic lateral sclerosis in SOD1 mice. Front. Cell. Neurosci. 10, 117.
Juranek, J.K., Daffu, G.K., Wojtkiewicz, J., Lacomis, D., Kofler, J., Schmidt, A.M., 2015. Receptor for advanced glycation end products and its inflammatory ligands are up-regulated in amyotrophic lateral sclerosis. Front. Cell. Neurosci. 9, 485.
Kamide, T., Kitao, Y., Takeichi, T., Okada, A., Mohri, H., Schmidt, A.M., Kawano, T., Munesue, S., Yamamoto, Y., Yamamoto, H., Hamada, J., Hori, O., 2012. RAGE mediates vascular injury and inflammation after global cerebral ischemia. Neurochem. Int. 60, 220–228.
Khan, M.A., Schultz, S., Othman, A., Fleming, T., Lebron-Galan, R., Rades, D., Clemente, D., Nawroth, P.P., Schwaninger, M., 2016. Hyperglycemia in stroke impairs polarization of monocytes/macrophages to a protective noninflammatory cell type. J. Neurosci. 36, 9313–9325.
Kim, M.J., Vargas, M.R., Harlan, B.A., Killoy, K.M., Ball, L.E., Comte-Walters, S., Gooz, M., Yamamoto, Y., Beckman, J.S., Barbeito, L., Pehar, M., 2018. Nitration and glycation turn mature NGF into a toxic factor for motor neurons: a role for $p75^{NTR}$ and RAGE signaling in ALS. Antioxidants Redox Signal. 28, 1587–1602.
Kisler, K., Nelson, A.R., Montagne, A., Zlokovic, B.V., 2017. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. Nat. Rev. Neurosci. 18, 419–434.
Koch, M., Chitayat, S., Dattilo, B.M., Schiefner, A., Diez, J., Chazin, W.J., Fritz, G., 2010. Structural basis for ligand recognition and activation of RAGE. Structure 18, 1342–1352.
Kook, S.Y., Hong, H.S., Moon, M., Ha, C.M., Chang, S., Mook-Jung, I., 2012. $A\beta_{1-42}$-RAGE interaction disrupts tight junctions of the blood-brain barrier via Ca(2)(+)-calcineurin signaling. J. Neurosci. 32, 8845–8854.
Kühn, S., Geyer, M., 2014. Formins as effector proteins of Rho GTPases. Small GTPases 5, e29513.
Kumano-Kuramochi, M., Ohnishi-Kameyama, M., Xie, Q., Niimi, S., Kubota, F., Komba, S., Machida, S., 2009. Minimum stable structure of the receptor for advanced glycation end product possesses multi ligand binding ability. Biochem. Biophys. Res. Commun. 386, 130–134.
Landis, C.A., Collins, B.J., Cribbs, L.L., Sukhatme, V.P., Bergmann, B.M., Rechtschaffen, A., Smalheiser, N.R., 1993. Expression of Egr-1 in the brain of sleep deprived rats. Brain Res Mol Brain Res 17, 300–306.
Leclerc, E., Fritz, G., Vetter, S.W., Heizmann, C.W., 2009. Binding of S100 proteins to RAGE: an update. Biochim. Biophys. Acta 1793, 993–1007.
Lee, J., Bayarsaikhan, D., Arivazhagan, R., Park, H., Lim, B., Gwak, P., Jeong, G.B., Lee, J., Byun, K., Lee, B., 2019. CRISPR/Cas9 edited sRAGE-MSCs protect neuronal death in Parkinson's disease model. Int J Stem Cells. https://doi.org/10.15283/ijsc18110.
Lei, C., Zhang, S., Cao, T., Tao, W., Liu, M., Wu, B., 2015. HMGB1 may act via RAGE to promote angiogenesis in the later phase after intracerebral hemorrhage. Neuroscience 295, 39–47.
Li, H., Wu, W., Sun, Q., Liu, M., Li, W., Zhang, X.S., Zhou, M.L., Hang, C.H., 2014. Expression and cell distribution of receptor for advanced glycation end-products in the rat cortex following experimental subarachnoid hemorrhage. Brain Res. 1543, 315–323.
Li, H., Yu, J.S., Zhang, D.D., Yang, Y.Q., Huang, L.T., Yu, Z., Chen, R.D., Yang, H.K., Hang, C.H., 2017a. Inhibition of the receptor for advanced glycation end-products (RAGE) attenuates neuroinflammation while sensitizing cortical neurons towards death in experimental subarachnoid hemorrhage. Mol. Neurobiol. 54, 755–767.
Li, Y., Zhu, J., Chen, L., Hu, W., Wang, M., Li, S., Gu, X., Tao, H., Zhao, B., Ma, G., Li, K., 2017b. Genetic predisposition to ischaemic stroke by RAGE and HMGB1 gene variants in Chinese Han population. Oncotarget 8, 100150–100164.
Liu, L.P., Hong, H., Liao, J.M., Wang, T.S., Wu, J., Chen, S.S., Li, Y.Q., Long, Y., Xia, Y.Z., 2009. Upregulation of RAGE at the blood-brain barrier in streptozotocin-induced diabetic mice. Synapse 63, 636–642.
Liu, W., Ge, S., Liu, Y., Wei, C., Ding, Y., Chen, A., Wu, Q., Zhang, Y., 2015. Polymorphisms in three genes are associated with hemorrhagic stroke. Brain Behav 5, e00395.
Loomis, S.J., Chen, Y., Sacks, D.B., Christenson, E.S., Christenson, R.H., Rebholz, C.M., Selvin, E., 2017. Cross-sectional analysis of AGE-CML, sRAGE, and esRAGE with diabetes and cardiometabolic risk factors in a community-based cohort. Clin. Chem. 63, 980–989.
Lopez-Diez, R., Shekhtman, A., Ramasamy, R., Schmidt, A.M., 2016. Cellular mechanisms and consequences of glycation in atherosclerosis and obesity. Biochim. Biophys. Acta 1862, 2244–2252.
Lue, L.F., Walker, D.G., Jacobson, S., Sabbagh, M., 2009. Receptor for advanced glycation end products: its role in Alzheimer's disease and other neurological diseases. Future Neurol. 4, 167–177.
Lyon, M.S., Wosiski-Kuhn, M., Gillespie, R., Caress, J., Milligan, C., 2019. Inflammation, Immunity, and amyotrophic lateral sclerosis: I. Etiology and pathology. Muscle Nerve 59, 10–22.
Maggioli, E., Boiocchi, C., Zorzetto, M., Sinforiani, E., Cereda, C., Ricevuti, G., Cuccia, M., 2013. The human leukocyte antigen class III haplotype approach: new insight in Alzheimer's disease inflammation hypothesis. Curr. Alzheimer Res. 10, 1047–1056.
Manigrasso, M.B., Friedman, R.A., Ramasamy, R., D'Agati, V.D., Schmidt, A.M., 2018. Deletion of the formin, Diaph1, protects from structural and functional abnormalities in the murine diabetic kidney. Am. J. Physiol. Renal. Physiol. 315 (6), 1601–1612.
Manigrasso, M.B., Pan, J., Rai, V., Zhang, J., Reverdatto, S., Quadri, N., DeVita, R.J., Ramasamy, R., Shekhtman, A., Schmidt, A.M., 2016. Small molecule inhibition of ligand-stimulated RAGE-DIAPH1 signal transduction. Sci. Rep. 6, 22450.
Mei, X., Wang, H., Zhang, H., Liu, C., Guo, Z., Wang, Y., Yuan, Y., Zhao, Z., Li, D., Tang, P., 2019. Blockade of receptor for advanced glycation end products promotes oligodendrocyte autophagy in spinal cord injury. Neurosci. Lett. 698, 198–203.
Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., Harrington, M.G., Chui, H.C., Law, M., Zlokovic, B.V., 2015. Blood-brain barrier breakdown in the aging human hippocampus. Neuron 85, 296–302.
Montaner, J., Mendioroz, M., Delgado, P., Garcia-Berrocoso, T., Giralt, D., Merino, C., Ribo, M., Rosell, A., Penalba, A., Fernandez-Cadenas, I., Romero, F., Molina, C., Alvarez-Sabin, J., Hernandez-Guillamon, M., 2012. Differentiating ischemic from hemorrhagic stroke using plasma biomarkers: the S100B/RAGE pathway. J Proteomics 75, 4758–4765.
Moser, B., Szabolcs, M.J., Ankersmit, H.J., Lu, Y., Qu, W., Weinberg, A., Herold, K.C., Schmidt, A.M., 2007. Blockade of RAGE suppresses alloimmune reactions in vitro and delays allograft rejection in murine heart transplantation. Am. J. Transplant. 7, 293–302.
Muhammad, S., Barakat, W., Stoyanov, S., Murikinati, S., Yang, H., Tracey, K.J., Bendszus, M., Rossetti, G., Nawroth, P.P., Bierhaus, A., Schwaninger, M., 2008. The HMGB1 receptor RAGE mediates ischemic brain damage. J. Neurosci. 28, 12023–12031.
Nation, D.A., Sweeney, M.D., Montagne, A., Sagare, A.P., D'Orazio, L.M., Pachicano, M., Sepehrband, F., Nelson, A.R., Buennagel, D.P., Harrington, M.G., Benzinger, T.L.S., Fagan, A.M., Ringman, J.M., Schneider, L.S., Morris, J.C., Chui, H.C., Law, M., Toga, A.W., Zlokovic, B.V., 2019. Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat. Med. 25, 270–276.
O'Shea, K.M., Ananthakrishnan, R., Li, Q., Quadri, N., Thiagarajan, D., Sreejit, G., Wang, L., Zirpoli, H., Aranda, J.F., Alberts, A.S., Schmidt, A.M., Ramasamy, R., 2017. The formin, DIAPH1, is a key modulator of myocardial ischemia/reperfusion injury. EBioMedicine 26, 165–174.
Olsson, S., Jood, K., 2013. Genetic variation in the receptor for advanced glycation end-products (RAGE) gene and ischaemic stroke. Eur. J. Neurol. 20, 991–993.
Palanissami, G., Paul, S.F.D., 2018. RAGE and its ligands: molecular interplay between glycation, inflammation, and hallmarks of cancer-a review. Horm Cancer 9, 295–325.
Park, H., Adsit, F.G., Boyington, J.C., 2010. The 1.5 Å crystal structure of human receptor for advanced glycation endproducts (RAGE) ectodomains reveals unique features determining ligand binding. J. Biol. Chem. 285, 40762–40770.
Park, M.H., Lee, J.Y., Park, K.H., Jung, I.K., Kim, K.T., Lee, Y.S., Ryu, H.H., Jeong, Y., Kang, M., Schwaninger, M., Gulbins, E., Reichel, M., Kornhuber, J., Yamaguchi, T., Kim, H.J., Kim, S.H., Schuchman, E.H., Jin, H.K., Bae, J.S., 2018. Vascular and neurogenic rejuvenation in aging mice by modulation of ASM. Neuron 100, 762.
Pinto, S., Cunha, C., Barbosa, M., Vaz, A.R., Brites, D., 2017. Exosomes from NSC-34 cells transfected with hSOD1-G93A are enriched in mir-124 and drive alterations in microglia phenotype. Front. Neurosci. 11, 273.
Presta, I., Vismara, M., Novellino, F., Donato, A., Zaffino, P., Scali, E., Pirrone, K.C., Spadea, M.F., Malara, N., Donato, G., 2018. Innate immunity cells and the neurovascular unit. Int. J. Mol. Sci. 19.
Rai, V., Maldonado, A.Y., Burz, D.S., Reverdatto, S., Yan, S.F., Schmidt, A.M., Shekhtman, A., 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, 5133–5144.
Ramasamy, R., Shekhtman, A., Schmidt, A.M., 2016. The multiple faces of RAGE–opportunities for therapeutic intervention in aging and chronic disease. Expert Opin. Ther. Targets 20, 431–446.
Raucci, A., Cugusi, S., Antonelli, A., Barabino, S.M., Monti, L., Bierhaus, A., Reiss, K., Saftig, P., Bianchi, M.E., 2008. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). FASEB J. 22, 3716–3727.
Ruiz, H.H., Chi, T., Shin, A.C., Lindtner, C., Hsieh, W., Ehrlich, M., Gandy, S., Buettner, C., 2016. Increased susceptibility to metabolic dysregulation in a mouse model of Alzheimer's disease is associated with impaired hypothalamic insulin signaling and

elevated BCAA levels. Alzheimers Dement 12, 851–861.
Saito, R., Araki, S., Yamamoto, Y., Kusuhara, K., 2017. Elevated endogenous secretory receptor for advanced glycation end products (esRAGE) levels are associated with circulating soluble RAGE levels in diabetic children. J. Pediatr. Endocrinol. Metab. 30, 63–69.
Sasaki, N., Takeuchi, M., Chowei, H., Kikuchi, S., Hayashi, Y., Nakano, N., Ikeda, H., Yamagishi, S., Kitamoto, T., Saito, T., Makita, Z., 2002. Advanced glycation end products (AGE) and their receptor (RAGE) in the brain of patients with Creutzfeldt-Jakob disease with prion plaques. Neurosci. Lett. 326, 117–120.
Schmidt, A.M., 2015. Soluble RAGEs - prospects for treating & tracking metabolic and inflammatory disease. Vasc. Pharmacol. 72, 1–8.
Schmidt, A.M., Hasu, M., Popov, D., Zhang, J.H., Chen, J., Yan, S.D., Brett, J., Cao, R., Kuwabara, K., Costache, G., et al., 1994. Receptor for advanced glycation end products (AGEs) has a central role in vessel wall interactions and gene activation in response to circulating AGE proteins. Proc. Natl. Acad. Sci. U. S. A. 91, 8807–8811.
Serrano, A., Donno, C., Giannetti, S., Peric, M., Andjus, P., D'Ambrosi, N., Michetti, F., 2017. The astrocytic S100B protein with its receptor RAGE is aberrantly expressed in SOD1^{G93A} models, and its inhibition decreases the expression of proinflammatory genes. Mediat. Inflamm. 2017, 1626204.
Shang, L., Ananthakrishnan, R., Li, Q., Quadri, N., Abdillahi, M., Zhu, Z., Qu, W., Rosario, R., Toure, F., Yan, S.F., Schmidt, A.M., Ramasamy, R., 2010. RAGE modulates hypoxia/reoxygenation injury in adult murine cardiomyocytes via JNK and GSK-3beta signaling pathways. PLoS One 5, e10092.
Sokol, B., Wasik, N., Jankowski, R., Holysz, M., Manko, W., Juszkat, R., Malkiewicz, T., Jagodzinski, P.P., 2017. Increase of soluble RAGE in cerebrospinal fluid following subarachnoid haemorrhage. BioMed Res. Int. 2017, 7931534.
Sorci, G., Riuzzi, F., Agneletti, A.L., Marchetti, C., Donato, R., 2003. S100B inhibits myogenic differentiation and myotube formation in a RAGE-independent manner. Mol. Cell Biol. 23, 4870–4881.
Sweeney, M.D., Sagare, A.P., Zlokovic, B.V., 2018. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat. Rev. Neurol. 14, 133–150.
Takeshita, Y., Shibata, N., Kasanuki, K., Nagata, T., Shinagawa, S., Kobayashi, N., Ohnuma, T., Suzuki, A., Kawai, E., Takayama, T., Nishioka, K., Motoi, Y., Hattori, N., Nakayama, K., Yamada, H., Arai, H., 2017. Genetic association between RAGE polymorphisms and Alzheimer's disease and Lewy body dementias in a Japanese cohort: a case-control study. Int. J. Geriatr. Psychiatry 32, 1241–1246.
Tang, S.C., Yang, K.C., Hu, C.J., Chiou, H.Y., Wu, C.C., Jeng, J.S., 2017. Elevated plasma level of soluble form of RAGE in ischemic stroke patients with dementia. NeuroMolecular Med. 19, 579–583.
Tanji, N., Markowitz, G.S., Fu, C., Kislinger, T., Taguchi, A., Pischetsrieder, M., Stern, D., Schmidt, A.M., D'Agati, V.D., 2000. Expression of advanced glycation end products and their cellular receptor RAGE in diabetic nephropathy and nondiabetic renal disease. J. Am. Soc. Nephrol. 11, 1656–1666.
Teismann, P., Sathe, K., Bierhaus, A., Leng, L., Martin, H.L., Bucala, R., Weigle, B., Nawroth, P.P., Schulz, J.B., 2012. Receptor for advanced glycation endproducts (RAGE) deficiency protects against MPTP toxicity. Neurobiol. Aging 33, 2478–2490.
Tian, X., Sun, L., Feng, D., Sun, Q., Dou, Y., Liu, C., Zhou, F., Li, H., Shen, H., Wang, Z., Chen, G., 2017. HMGB1 promotes neurovascular remodeling via Rage in the late phase of subarachnoid hemorrhage. Brain Res. 1670, 135–145.
Touré, F., Fritz, G., Li, Q., Rai, V., Daffu, G., Zou, Y.S., Rosario, R., Ramasamy, R., Alberts, A.S., Yan, S.F., Schmidt, A.M., 2012. Formin mDia1 mediates vascular remodeling via integration of oxidative and signal transduction pathways. Circ. Res. 110, 1279–1293.
Van Dyken, P., Lacoste, B., 2018. Impact of metabolic syndrome on neuroinflammation and the blood-brain barrier. Front. Neurosci. 12, 930.
Villegas-Llerena, C., Phillips, A., Garcia-Reitboeck, P., Hardy, J., Pocock, J.M., 2016. Microglial genes regulating neuroinflammation in the progression of Alzheimer's disease. Curr. Opin. Neurobiol. 36, 74–81.
VTV Therapeutics, 2018. http://ir.vtvtherapeutics.com/phoenix.zhtml?c=254081&p=irol-newsArticle&ID=2341681.
Wan, W., Cao, L., Liu, L., Zhang, C., Kalionis, B., Tai, X., Li, Y., Xia, S., 2015. Aβ(1-42) oligomer-induced leakage in an in vitro blood-brain barrier model is associated with up-regulation of RAGE and metalloproteinases, and down-regulation of tight junction scaffold proteins. J. Neurochem. 134, 382–393.
Wang, H., Mei, X., Cao, Y., Liu, C., Zhao, Z., Guo, Z., Bi, Y., Shen, Z., Yuan, Y., Guo, Y., Song, C., Bai, L., Wang, Y., Yu, D., 2017a. HMGB1/Advanced Glycation End Products (RAGE) does not aggravate inflammation but promote endogenous neural stem cells differentiation in spinal cord injury. Sci. Rep. 7, 10332.
Wang, H., Zhao, Z., Liu, C., Guo, Z., Yuan, Y., Zhao, H., Zhou, Z., Mei, X., 2018. Receptor for advanced glycation end-products (RAGE) blockade do damage to neuronal survival via disrupting Wnt/beta-catenin signaling in spinal cord injury. Neurochem. Res. 43, 1405–1412.
Wang, P., Huang, R., Lu, S., Xia, W., Cai, R., Sun, H., Wang, S., 2016. RAGE and AGEs in mild cognitive impairment of diabetic patients: a cross-sectional study. PLoS One 11 e0145521.
Wang, Z., Wang, S.N., Xu, T.Y., Miao, Z.W., Su, D.F., Miao, C.Y., 2017b. Organoid technology for brain and therapeutics research. CNS Neurosci. Ther. 23, 771–778.
Wang, Z.X., Wan, Y., Tan, L., Liu, J., Wang, H.F., Sun, F.R., Tan, M.S., Tan, C.C., Jiang, T., Tan, L., Yu, J.T., 2017c. Genetic association of HLA gene variants with MRI brain structure in alzheimer's disease. Mol. Neurobiol. 54, 3195–3204.
Wautier, J.L., Zoukourian, C., Chappey, O., Wautier, M.P., Guillausseau, P.J., Cao, R., Hori, O., Stern, D., Schmidt, A.M., 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, 238–243.
Wu, X., Wu, J., Thompson, C.W., Li, Y., 2015. Adaptive evolution of the MHC class III-encoded receptor RAGE in primates and murine rodents. Int. J. Immunogenet. 42, 461–468.
Xie, J., Burz, D.S., He, W., Bronstein, I.B., Lednev, I., Shekhtman, A., 2007. Hexameric calgranulin C (S100A12) binds to the receptor for advanced glycated end products (RAGE) using symmetric hydrophobic target-binding patches. J. Biol. Chem. 282, 4218–4231.
Xie, J., Reverdatto, S., Frolov, A., Hoffmann, R., Burz, D.S., Shekhtman, A., 2008. Structural basis for pattern recognition by the receptor for advanced glycation end products (RAGE). J. Biol. Chem. 283, 27255–27269.
Xu, W.L., Qiu, C.X., Wahlin, A., Winblad, B., Fratiglioni, L., 2004. Diabetes mellitus and risk of dementia in the Kungsholmen project: a 6-year follow-up study. Neurology 63, 1181–1186.
Xu, Y., Toure, F., Qu, W., Lin, L., Song, F., Shen, X., Rosario, R., Garcia, J., Schmidt, A.M., Yan, S.F., 2010. Advanced glycation end product (AGE)-receptor for AGE (RAGE) signaling and up-regulation of Egr-1 in hypoxic macrophages. J. Biol. Chem. 285, 23233–23240.
Xue, J., Manigrasso, M., Scalabrin, M., Rai, V., Reverdatto, S., Burz, D.S., Fabris, D., Schmidt, A.M., Shekhtman, A., 2016. Change in the molecular dimension of a RAGE-ligand complex triggers RAGE signaling. Structure 24, 1509–1522.
Yan, S.D., Chen, X., Fu, J., Chen, M., Zhu, H., Roher, A., Slattery, T., Zhao, L., Nagashima, M., Morser, J., Migheli, A., Nawroth, P., Stern, D., Schmidt, A.M., 1996. RAGE and amyloid-beta peptide neurotoxicity in Alzheimer's disease. Nature 382, 685–691.
Yang, F., Wang, Z., Zhang, J.H., Tang, J., Liu, X., Tan, L., Huang, Q.Y., Feng, H., 2015. Receptor for advanced glycation end-product antagonist reduces blood-brain barrier damage after intracerebral hemorrhage. Stroke 46, 1328–1336.
Ye, X., Chopp, M., Liu, X., Zacharek, A., Cui, X., Yan, T., Roberts, C., Chen, J., 2011. Niaspan reduces high-mobility group box 1/receptor for advanced glycation end-products after stroke in type-1 diabetic rats. Neuroscience 190, 339–345.
Yonekura, H., Yamamoto, Y., Sakurai, S., Petrova, R.G., Abedin, M.J., Li, H., Yasui, K., Takeuchi, M., Makita, Z., Takasawa, S., Okamoto, H., Watanabe, T., Yamamoto, H., 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, 1097–1109.
Zhang, Z.G., Li, Y., Ng, C.T., Song, Y.Q., 2015. Inflammation in alzheimer's disease and molecular genetics: recent update. Arch. Immunol. Ther. Exp. 63, 333–344.
Zhou, X., Weng, J., Xu, J., Xu, Q., Wang, W., Zhang, W., Huang, Q., Guo, X., 2018. Mdia1 is crucial for advanced glycation end product-induced endothelial hyperpermeability. Cell. Physiol. Biochem. 45, 1717–1730.