Receptor for advanced glycation end-products-mediated inflammation and diabetic vascular complications

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Exposure of amino residue of proteins to reducing sugars, such as glucose, glucose 6-phosphate, fructose, ribose and intermediate aldehydes, results in non-enzymatic glycation, which forms reversible Schiff bases and Amadori compounds. A series of further complex molecular rearrangements then yield irreversible advanced glycation end-products (AGE). The aldehydes, highly reactive AGE precursors, are produced by both enzymatic and non-enzymatic pathways. The enzymatic pathways include a route of myeloperoxidase in inflammatory cells, such as activated macrophages, which produces hypochlorite, then reacting with serine to generate glycolaldehyde. Methylglyoxal (MGO) is generated by the degeneration of triose phosphate intermediates in the glycolytic system. 3-Deoxyglucosone (3DG) is produced by fructosamine-3-kinase from fructoselysine, an Amadori compound. Another route is the formation of aldehydes, glucosone and glyoxal from peroxynitrite ($ONOO^-$) and superoxide anion radical ($O_2^{-}\cdot$). Non-enzymatic pathways include glucose autoxidation resulting in the formation of glyoxal and hydrolysis of Amadori rearrangement products yielding 3DG. In diabetes, uncontrolled and sustained hyperglycemia superdrives this glycation reaction, and AGE accumulate in the circulation and various tissues. A hypothesis that interactions between AGE and the receptor for AGE (RAGE) are the crucial cause of diabetic vascular derangements has emerged from experiments with vascular endothelial cells (EC), pericytes and renal mesangial cells in culture and from AGE inhibitor-treated or RAGE-gene manipulated animals[1,2]. Among a number of AGE components, carboxylmethyllysine (CML) and AGE generated from glyceraldehyde or glycolaldehyde were found to bind RAGE and subsequently elicit RAGE signaling.

RAGE consists of an extracellular region, a transmembrane domain and a 43-amino acid short cytoplasmic tail. The extracellular region is composed of three immunoglobulin-like domains: one V-type and two C-type domains. The V-type domain of RAGE was found to interact with AGE, and its lack of *N*-glycosylation modification enhanced the binding affinity to AGE. The possible mechanism of the binding includes charge association and subsequent stabilization of the complex with hydrophobic interaction after conformational changes of RAGE V-type domain[3]. This concept is supported by the fact that low molecular weight heparin (LMWH; approximately 5 kDa and negative charged) binds RAGE and inhibits AGE–RAGE association, resulting in silencing RAGE activation of NFκB[2]. In EC, RAGE signaling caused by AGE could induce expressions of genes for vascular endothelial growth factor (VEGF) and for vascular cell adhesion molecule-1 (VCAM-1), leading to enhancement of vascular permeability, angiogenesis, and local inflammation. The endothelial–mesenchymal transition (EndoMT) was also induced by this system[4]. Secretion of various cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and monocyte chemotactic protein-1 (MCP-1), were induced by the AGE–RAGE signaling in monocytes and macrophages. RAGE promoter assays showed that RAGE signaling by AGE promoted the transcriptional upregulation of the RAGE gene by itself through an activation of NFκB. TNF-α and estrogen also enhanced a transcription of the *RAGE* gene through an activation of NFκB and a transcription factor Sp1, respectively. Recently, the mammalian homolog of the *Drosophila* gene, diaphanous 1 (mDia1), has been identified as a direct binding molecule with an intracellular domain of RAGE and as a part of the machinery of RAGE intracellular signaling. mDia1, one of the formin homology proteins, exists widely from yeast to mammals and is known to link with cell division, polarity formation and movements by actin polymerization.

Because other pro-inflammatory ligands of RAGE have been identified, including high-mobility group box protein 1 (HMGB1), advanced oxidation protein products (AOPP), S100-calcium binding (S100) proteins, CD11b (Mac-1), amyloid β-proteins and complement C3a, lipopolysaccharides (LPS), and phosphatidylserine on the surface of apoptotic cells, RAGE is considered a member of pattern recognition receptors (PRR), such as toll-like receptors (TLR), actively participating in inflammation, diabetic vascular complications and atherosclerosis (Figure 1). HMGB1 is a nuclear protein stabilizing nucleosome formation and facilitating transcription. Though HMGB1 lacks a classical secretion signal, it is known as a strong inflammatory mediator from necrotic cells by passive leakage and can be actively secreted by activated monocytes, macrophages, dendritic cells (DC), natural killer (NK) cells and EC. HMGB1 association with RAGE is enhanced by the presence of CpG DNA, and HMGB1 directly binds LPS and IL-1β. The formation of the complex with other pro-inflammatory molecules further aggravates activation of RAGE signaling. TLR2 and TLR4 have also been identified to be involved in HMGB1-induced cellular signaling. Hence, the interplay of RAGE/TLR receptors and their signaling pathways exacerbates NFκB activation and pro-inflammatory reactions. As anti-inflammatory machinery, the lectin domain of thrombomodulin (TM) can bind HMGB1 and

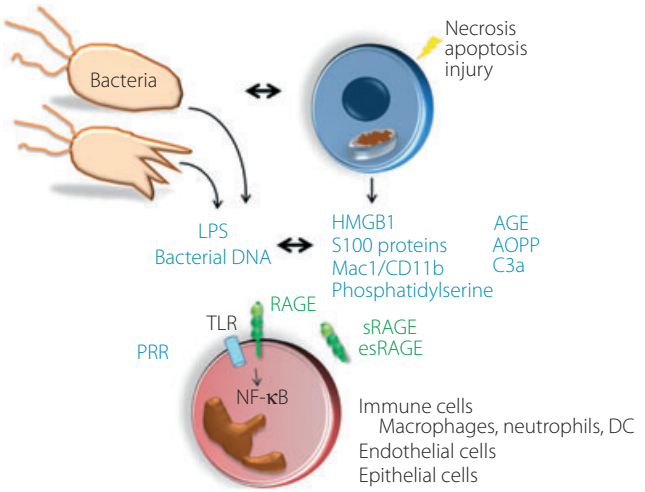

Figure 1 | Receptor for advanced glycation end-products (RAGE) as a member of pattern-recognition receptors (PRR) participating in inflammation and diabetic vascular complications. AGE, advanced glycation end-products; AOPP, advanced oxidation protein products; esRAGE, endogenous secretory receptor for advanced glycation end-products; HMGB1, high-mobility group box protein 1; LPS, lipopolysaccharides; sRAGE, soluble receptor for advanced glycation end-products; TLR, toll-like receptors.

thereby blocks HMGB1 interaction with RAGE. S100 proteins are a family of over 20 proteins sharing structural similarity with their two EF-hand Ca^{++} binding domains flanked by α-helices; among them, S100A1, A2, A4, A5, A6, A7/A7A, A8/A9, A11, A12, B and P could bind RAGE. Their higher oligomerization states lead to RAGE activation. A recent report showed CML-modified S100A8/A9 strongly activated intestinal inflammatory responses through RAGE, suggesting more complex varieties of RAGE ligands modified by glycation reactions. Although deglycosylation sensitizes RAGE to bind AGE, carboxylated *N*-glycans on RAGE increase the binding affinity with S100A8/9, as well as HMGB1. S100A8/A9 was also shown to interact with TLR4. CD11b (Mac-1) is a cell surface molecule expressed on neutrophils, monocytes, macrophages, dendritic cells and NK cells. It mediates the interaction of RAGE together with endothelial counter-receptors, such as intercellular adhesion molecule 1 (ICAM-1). With this adhesion, RAGE thus mediates recruitment and accumulation of the immune cells into inflammatory foci. The activation of RAGE on T cells leads to the differentiation of Th1 cells, thus playing a role in balancing Th1 and Th2 immunity. RAGE triggers the maturation of DC to migrate to draining lymph nodes.

It is reported that the binding of HMGB1 to RAGE induces RAGE shedding by a disintegrin and metalloproteinase 10 (ADAM10), as well as an intracellular signal transduction, representing one pathway for autodownregulation of RAGE-mediated cellular activation. The cleavage of the membrane-bound full-length RAGE (signal transducer) yields soluble RAGE (sRAGE), which could work as a decoy receptor against ligand-RAGE interactions. In the strict sense of the word, sRAGE is a heterogeneous population of total sRAGE proteins, including soluble splice variants of RAGE and the proteinase-cleaved forms of membrane-bound RAGE and of the soluble variants. Endogenous secretory RAGE (esRAGE)[5] is one of the major splice variants of RAGE existing in the circulation and also being widely distributed to the cell surface and cytoplasm of neurons, EC, pneumocytes, mesothelium, pancreatic β-cells, monocytes, macrophages, salivary glands, digestive tracts, renal tubules, prostate, skin, thyroid and bronchioles. The sRAGE is thought to act locally and systemically as a decoy receptor. The administration of sRAGE has been shown to attenuate experimental animal models of various RAGE-related diseases, such as atherosclerosis. sRAGE/esRAGE could also modulate inflammatory reactions. Reinforcing the ectodomain shedding will decrease a total amount and expression of full-length membrane-bound RAGE and will reciprocally increase an amount of sRAGE, this can control AGE-RAGE signaling and subsequent cellular and tissue derangement. Recent clinical studies have focused on the significance of circulating sRAGE in a variety of pathophysiological conditions. Above all, findings in both type 1 and type 2 diabetic patients are quite confusing, and have been reported to be both increased and decreased. The presence of renal insufficiency can affect circulating sRAGE level, which might explain the controversial findings of sRAGE in diabetes.

Considered together, the suppression of RAGE action might be beneficial for preventing and slowing down the development of diabetic vascular complications, atherosclerosis and inflammation. Potential candidates for this purpose might include a downregulator of full-length membrane-bound RAGE, RAGE-specific antagonists, blockers against RAGE intracellular signaling, enhancers of esRAGE production, supplementation of esRAGE or sRAGE and an inducer of RAGE shedding. Angiotensin-converting enzyme inhibitor, thiazolidine, and statin are reported to stimulate esRAGE/sRAGE secretion. LMWH, the depolymerized and fractionated derivative of heparin, has been shown to work as a RAGE antagonist by inhibiting AGE-RAGE signaling[2]. At present, TTP488 is a specific RAGE antagonist under clinical trials in the USA and its efficacy is anticipated. It will be interesting in the future to develop new devices and remedies that control the RAGE ectodomain shedding.

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