Roles of the Receptor for Advanced Glycation Endproducts in Diabetes-Induced Vascular Injury

Hideto Yonekura1,*, Yasuhiko Yamamoto1, Shigeru Sakurai1, Takuo Watanabe1, and Hiroshi Yamamoto1

1Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8640, Japan

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Abstract. Diabetic patients have shorter life span and poorer Quality of Life mainly due to diabetic vascular complications. Recent in vitro and in vivo studies have shown that advanced glycation endproducts (AGE) account for diabetic vascular complications through their engagement of the receptor for AGE (RAGE). In this review, we summarize our recent studies on the roles of the AGE-RAGE system in diabetes-induced vascular injury. In vitro experiments showed that AGE engagement of RAGE leads to changes in endothelial cells (EC) and pericytes, which are characteristic of diabetic microangiopathy. Diabetic RAGE transgenic mice that overexpress RAGE in vascular cells exhibited the exacerbation of the indices of nephropathy and retinopathy, and this was prevented by the inhibition of AGE formation. RAGE overexpression also caused calcium handling impairment in cardiac myocytes. In contrast to the RAGE-overexpressing mice, diabetic RAGE knockout mice showed marked improvement of nephropathy. We found that human vascular cells express a novel splice variant coding for a soluble RAGE protein and named it endogenous secretory RAGE (esRAGE). The esRAGE neutralizes AGE actions on EC and is present in human sera. Individual variations in circulating esRAGE could be a determinant for individual differences in susceptibility or resistance to the development of diabetic vascular complications. The AGE-RAGE system should be, therefore, a candidate molecular target for overcoming diabetic vascular complications.

Keywords: diabetic vascular complication, advanced glycation endproduct (AGE), receptor for AGE (RAGE), transgenic mouse, endogenous secretory RAGE

Introduction

Diabetes is a disease characterized by chronic hyperglycemia due to deficiency in insulin action. The population of diabetic patients is dramatically increasing in the world. The morbidity and mortality of diabetes are due to the development of both macrovascular and microvascular complications (1 – 6). The diabetic condition accelerates atherogenesis, myocardial infraction, and stroke. The diabetic condition also leads to the development of microvascular complications; three major diabetic microvascular complications are retinopathy, nephropathy, and neuropathy. Retinopathy is the leading cause of acquired blindness. Its typical changes are pericyte loss, thrombogenesis, and abnormal angiogenesis in the retina. Nephropathy is the most common cause of end-stage renal disease, and the patients with it have to receive dialysis. The major changes characteristic of nephropathy are enlargement of the kidney, glomerulosclerosis, proteinuria, and elevation in serum creatinine. The kidney lesion represents an accumulation of extracellular matrix proteins such as type IV collagen, which are produced by mesangial cells. More than half of diabetic patients develop neuropathy, a progressive deterioration of nerves, which results in peripheral and autonomic nerve dysfunction. Diabetic neuropathy, however, remains the least understood complication.

Studies over years have implicated several pathways of glucose metabolism in the development of vascular complications (1, 6): i) nonenzymatic glycation of pro-
teins yielding advanced glycation endproducts (AGE), which activate AGE receptor signaling; ii) increased polyol pathway activity; iii) activation of protein kinase C; iv) increased hexosamine pathway flux; and v) production of superoxide by the mitochondrial electron-transport chain.

Recent in vitro and animal experiments including those from our laboratory strongly suggested that AGE and their receptor (the receptor for AGE, RAGE) system takes a crucial part in development of diabetic vascular complications (2–7). In this review, we will focus on the roles of the AGE-RAGE system in diabetes-induced vascular injury and summarize recent studies mainly from our laboratory.

**AGE and RAGE**

Reducing sugars like glucose can react non-enzymatically with the amino groups of proteins to form reversible Schiff bases followed by Amadori rearrangement. These early glycation products undergo further complex reactions such as dehydration, condensation, and cross-linking to become irreversible heterogeneous derivatives termed advanced glycation endproducts (AGE) (3, 5, 8). AGE are known to accumulate in circulating blood and in various tissues at an extremely accelerated rate under the diabetic state and have been implicated in the development of diabetic vascular complications.

Receptor-dependent mechanisms are likely to work in the AGE-induced tissue dysfunction and the best-characterized AGE receptor is RAGE (the receptor for AGE) (2–5, 7, 9). RAGE is a multi-ligand cell surface receptor initially isolated from bovine lung as an AGE-binding protein by the group of Stern and Schmidt (10, 11). RAGE belongs to the immunoglobulin superfamily of cell surface molecules and is composed of an extracellular domain containing one “V”-type immunoglobulin domain and two “C”-type immunoglobulin domains (Fig. 1) (2, 9, 11). The extracellular portion of the receptor is followed by a hydrophobic transmembrane-spanning domain and then by a highly charged, short cytoplasmic domain that is essential for post-RAGE signaling (2, 9). Endogenous ligands, such as amphoterin, calgranulin, amyloid β proteins, and transthyretin, have also been identified (2, 9).

Previous studies have identified several downstream signaling pathways responsive to RAGE ligation (2, 5, 9). The engagement of RAGE by AGE has been reported to induce cellular oxidant stress, activating the transcription factor nuclear factor-κB (NF-κB) (2, 5, 9), resulting in the perturbation of a variety of homeostatic functions of the vasculature. RAGE-mediated NF-κB activation depends on activation of mitogen-activated protein kinase (MAPK) involving the small GTPase Ras and extracellular signal-regulated kinase 1 and 2 (ERK1/2). RAGE ligation has also been reported to activate p38 MAPK and stress-activated protein kinase c-Jun-N-terminal kinase (SAPK/JNK). The engagement of RAGE by amphoterin has been reported to activate Rho-family small GTPase Cdc42 and Rac1 (9). Signaling molecules binding directly to the RAGE cytoplasmic domain, however, remain unidentified, except that ERK1/2 have been recently reported to bind the membrane proximal region of the cytoplasmic domain (12).

In addition to glucose, short chain aldehydes including glycolaldehyde and glycoaldehyde, which are non-enzymically derived from glucose per se, can also produce AGE (3, 13). We conducted a surface plasmon resonance assay using purified recombinant human RAGE proteins and glucose- and aldehyde-derived AGE fractions (14). As a result, in addition to glucose-derived AGE, several aldehyde-derived AGE fractions were found to strongly bind to RAGE. These ligands were observed to elicit pericyte and endothelial cells (EC) derangement, suggesting that certain AGE structures affect vascular cells through interactions with RAGE (Y. Yamamoto et al., submitted for publication).

**AGE action on vascular cells in vitro**

We have shown previously that engagement of RAGE by AGE leads to changes in EC and pericytes, which are characteristic of diabetic microvascular complications (3, 7, 14–20) (Fig. 2). The group of Stern and Schmidt has also reported AGE-activation of EC (5, 21). AGE increased EC cell number in a dose-dependent manner (17) and induced expression of vascular endothelial growth factor (VEGF) in EC (14, 17, 19, 20) (see also Fig. 6B). VEGF is the most potent angiogenic factor and EC themselves produce it, which has an important role in EC growth regulation in an autocrine manner (22). It has been reported that AGE induce VEGF expression through activation of hypoxia-inducible factor-1 (HIF-1) activity (23). We have also demonstrated that AGE inhibit prostacyclin production and stimulate plasminogen activator inhibitor-1 (PAI-1) synthesis by EC (16, 18). AGE thus stimulate the growth of microvascular EC through an induction of VEGF, leading to angiogenesis on one hand, and inhibit prostacyclin production and stimulate PAI-1 synthesis by EC, thereby leading to thrombogenesis on the other (Fig. 2). AGE also exhibit toxic and growth inhibitory actions on pericytes (14, 15). Such a state does occur in the early phase of retinopathy and is known as “pericyte loss”, the earliest histological hallmark of diabetic retinopathy.
Fig. 1. Schematic representation of RAGE.

Fig. 2. Vascular and mesangial cell changes caused by AGE-RAGE interactions. Modified from Ref. 6.

Fig. 3. Albuminuria (A) and serum creatinine (B) levels in RAGE-overexpressing diabetic mice. Modified from Ref. 28.

Fig. 4. Renal histology (A) and sclerosis index (B) in RAGE-overexpressing diabetic mice. Modified from Ref. 28.
Pericytes not only regulate the growth of neighboring EC but also preserve EC-specific functions including the production of prostacyclin, an anti-thrombogenic prostanoïd (24). This indicates that when the pericyte-EC interaction is impaired, angiogenesis and thrombogenesis should be further accelerated (Fig. 2). Concerning nephropathy, the engagement of RAGE by AGE has reported to induce type IV collagen synthesis by glomerular mesangial cells, the pericyte equivalent in kidney glomerulus (Fig. 2), and it was prevented by a ribozyme against RAGE mRNA (25).

We have also demonstrated that AGE themselves upregulate the RAGE expression in microvascular EC through the activation of NF-κB (19). The engagement of RAGE by AGE induces expression of vascular cell adhesion molecule-1 (VCAM-1) in cultured human EC (21). RAGE has been also reported to be involved in leukocyte adhesion to EC (26) and monocyte transendothelial migration (27). Those activities may induce immune/inflammatory responses, leading to aggravation of diabetic vascular complications.

**RAGE transgenic mice**

From the in vitro experiments, the AGE-RAGE system would play an active part in the development of diabetic complications. To evaluate this idea in vivo, we then employed animal experiments. First we created transgenic mice that overexpress human RAGE protein in vascular cells (7, 28). Human RAGE gene was linked to mouse flk-1 (VEGF receptor 2) promoter, which works preferentially in vascular cells, and introduced into fertilized ova of mice. The RAGE transgenic mouse was cross-bred with a type 1 diabetes model mouse. This diabetes model mouse was created by introducing inducible nitric oxide synthase (iNOS) cDNA under the control of insulin promoter; the mice develop diabetes as early as 1 week after birth by NO-mediated destruction of insulin-producing islet β-cells (29). Cross-breeding between the two transgenic mice yielded four groups, and the resultant iNOS transgenic and the double transgenic mice were diabetic. Double and iNOS transgenic mice showed hyperglycemia and higher hemoglobin A1c levels, but there was no significant difference between the two diabetic groups (28). The first detectable change was enlargement of the kidney (nephromegaly) in the double transgenic mice (28). Albuminuria was evident in the double transgenic mice at 4 months of age (Fig. 3A). Figure 3B shows the serum creatinine level and it was significantly increased in double transgenic mice at 6 months of age. Figure 4A shows renal histology; the deposition of PAS-positive material in the mesangial area of double and iNOS transgenic mice were evident, and their glomeruli were diagnosed as glomerulosclerosis. Figure 4B shows quantitative evaluation of glomerulosclerosis; the highest scores were noted in the double transgenic mice. The increases in serum creatinine and sclerosis index were effectively prevented with (±)-2-isopropylidenedehydrorazo-4-oxothiazolidin-5-ylacetanilide, an inhibitor of AGE formation (28). This mouse model did not show very severe retinopathy, but indices diagnostic of diabetic retinopathy were also most prominent in the double transgenic mice, exemplified by increases in vascular permeability and avascular area in the retina (3).

We also created transgenic mice that express human RAGE in the heart and analyzed the Ca²⁺ transients in cultured cardiac myocytes from RAGE-transgenic and wild-type mice. RAGE overexpression reduced the systolic and diastolic intracellular calcium concentration (30). Although in vivo cardiovascular phenotypes of the RAGE transgenic mice remain to be tested, the results suggested that the AGE-RAGE system could play an active role in the development of diabetes-induced cardiac dysfunction.

**RAGE knockout mice**

We next created the RAGE knockout mouse. The first two exons of the mouse RAGE gene were sandwiched by lox-P elements and then were deleted by a treatment with Cre recombinase to create knockout mouse (Y. Yamamoto et al., submitted for publication). The RAGE knockout mouse was crossbred with the iNOS transgenic diabetic mouse, and the RAGE-deficient diabetic mice were then assayed for nephropathy. In contrast to the diabetic RAGE-overexpressing mice, the diabetic RAGE knockout mice showed marked improvement of nephromegaly, albuminuria, glomerulosclerosis, and serum creatinine level (summarized in Fig. 5; Y. Yamamoto et al., submitted for publication). Recently, significantly decreased neointimal expansion after arterial injury in RAGE null mice has been reported, indicating key roles for RAGE in modulating smooth muscle cell properties after injury (31).

**Endogenous secretory RAGE (esRAGE)**

To deepen our understanding of the physiology and pathology of RAGE and to develop effective means for the prophylaxis and therapy of diabetic vascular complications, it is important to elucidate the nature of RAGE proteins expressed in vascular cells. We thus cloned RAGE cDNAs using polysomal poly(A) RNA from human EC and pericytes and determined their structures and found that human vascular cells mainly express...
three RAGE splice variants (3, 20, 32). The first was the known full-length RAGE and the second encoded an amino-terminally truncated form of currently unknown function. The third encoded a novel carboxy-terminally truncated, soluble form (Fig. 1). Transfection experiments of cDNA in COS-7 cells showed that C-truncated type cDNA yielded 50-kDa glycosylated RAGE protein and it was secreted into culture medium (20). We also demonstrated that this soluble type RAGE protein is actually produced by primary cultured human EC and pericytes and is secreted into culture medium (20). We named this naturally occurring soluble RAGE protein “esRAGE (endogenous secretory RAGE)” (3, 20, 32). It has been reported that soluble RAGE protein artificially produced by recombinant gene technology can bind AGE and prevent the progression of diabetic vasculopathy and atherosclerosis in experimental animals (4, 33). The naturally occurring esRAGE also does bind to an AGE ligand and have an activity that neutralizes the AGE action (20). The addition of esRAGE blocked both AGE-induced ERK phosphorylation (Fig. 6A) and VEGF induction (Fig. 6B). The results indicated that esRAGE is cytoprotective against AGE actions.

Vascular complications and esRAGE

The levels of RAGE variant expressions may vary among individuals and/or conditions. We propose that such diversity could be a factor that endows diabetic patients with different susceptibility or resistance to the development of diabetic vascular complications. Thus, we next examined whether esRAGE occurs in vivo in humans and whether it is related to an individual’s resistance to the development of diabetic vasculopathy. As a result, we detected esRAGE in human sera from healthy subjects by immunoblotting with an esRAGE-specific antibody (3, 20, 32). We then developed a new, highly sensitive, and specific ELISA system for esRAGE and applied it to type 1 diabetic subjects and healthy controls (S. Sakurai et al., submitted for publication). The ELISA analysis showed that circulating esRAGE concentrations in the patients with simple and proliferative retinopathy were significantly lower than in those without retinopathy. The patient with the highest serum esRAGE level has not suffered from retinopathy during more than 10 years from the onset of diabetes.

Fig. 5. Diabetic nephropathy indices in RAGE knockout mice (Y. Yamamoto et al., submitted for publication).

Fig. 6. Effects of esRAGE on the AGE action. ERK phosphorylation (A) and VEGF mRNA expression (B) in EC. Modified from Ref. 20 (© the Biochemical Society) with permission.
The results suggested that the esRAGE confers protection against diabetic vascular complications.

Conclusions

The transgenic (28, 30) and knockout approaches (Y. Yamamoto et al., submitted for publication) have supported the concept that the AGE-RAGE system plays an active role in the development of diabetic nephropathy and retinopathy. These results also suggested that upregulation of RAGE gene expression accelerates the development of diabetic microvascular complications, and a decrease in RAGE gene expression makes diabetic patients relatively resistant to microangiopathy. RAGE has also been reported to be involved in the development of diabetic macrovascular complications and arterial injury (4, 31, 33).

The presence of esRAGE in human circulation should be of significance, since esRAGE would protect vascular cells from the activation of the cell surface receptor which would otherwise lead to vascular injury (3, 20, 32). Various genetic factors other than hyperglycemia are undoubtedly involved in the pathogenesis of diabetic vasculopathy. We assume that individual variations in circulating esRAGE could be a determinant for individual differences in susceptibility or resistance to the development of diabetic microvascular complications. Low esRAGE levels in the blood may be a risk for the development and progression of diabetic complications. The esRAGE may be used not only to predict the risk of diabetic vascular complications, but also to prevent them; if we could invent a means to selectively induce this splice variant, it would help increase resistance to the development of the disease.

The AGE-RAGE system thus is thought to play a central role in the development of diabetic vascular complications and to be a promising target for the treatment of this disease. The transgenic and knockout mice can be used as useful animal models for testing remedies.

References


