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Inhibitory effect of alternatively spliced RAGEv1 on the expression of NF-κB and TNF-α in hepatocellular carcinoma cells

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ABSTRACT. Binding of specific ligands to the receptor for advanced glycation end-products (RAGE) can trigger a series of signal transductions, which leads to pathogenesis in many chronic degenerative diseases, including cancer. Alternative splicing of RAGE mRNA has resulted in many variants, including RAGE variant 1 (RAGEv1). This particular splice variant of RAGE can provide a major soluble form of RAGE in blood circulation, which can neutralize deleterious ligands, thus diminishing signaling that can lead to inflammation and pathogenesis in cancer cells. However, the molecular mechanisms involved in suppressing signaling cascades in the cells are unknown. We investigated the molecular role of the RAGEv1 isoform in modulating NF-κB and TNF-α gene expression in human hepatocellular carcinoma HepG2 cells. Transient transfection using an engineered plasmid containing the RAGEv1 gene resulted in a significant increase in normalized RAGEv1 mRNA transcripts in HepG2 cells. This finding

was supported by the detection of the RAGEv1 protein, which was found in the whole-cell extracts and the cell culture media. This high degree of RAGEv1 expression significantly reduced the expression of normalized mRNA transcripts of NF-κB and TNF-α in HepG2 cells. We suggest that RAGEv1 could reduce activity of the NF-κB signaling pathway in liver cancer cells, thus providing a potential alternative therapy for the treatment of liver cancer.

Key words: RAGEv1; HepG2; NF-κB; TNF-α; Gene expression analysis

INTRODUCTION

The receptor for advanced glycation end-products (RAGE) is a protein in the immunoglobulin superfamily, which can bind not only AGEs (Neeper et al., 1992) but also other ligands such as S100/calgranulin (Hofmann et al., 1999) and high-mobility group box-1 (Hori et al., 1995). The human RAGE gene is composed of 11 exons and a short 3'-UTR, which is transcribed into an mRNA of 1.4 kb and translated to give a protein of 404 amino acids with a molecular mass of 55 kDa. However, the human RAGE gene has been reported to possess several alternatively spliced variants (Hudson et al., 2008). Interestingly, there has been a report of a unique splice variant of the RAGE gene, the second most prevalent RAGE isoform after full-length RAGE, occurring due to the alternative splicing of the inclusion of part of intron 9 and removal of exon 10 (Yonekura et al., 2003). This particular isoform has been demonstrated to cause an alteration in the reading frame sequence of the protein at amino acid 332, thus giving rise to the RAGE splice variant 1 (RAGEv1). Accordingly, this particular isoform of RAGE contains neither the transmembrane domain nor cytosolic domain. Previously, it has been referred to as endogenous secretory RAGE. RAGEv1 naturally occurs *in vivo* and was found in human blood circulation (Fernandez-Botran et al., 2002).

Binding of RAGE and ligand results in the activation of signal transduction cascade, thus leading to upregulation of RAGE and pro-inflammatory genes that are associated with the pathogenesis of chronic degenerative disorders (Schmidt et al., 2001). In addition, ligand-RAGE binding can activate signaling pathways, which play a causative role in the alteration of cancer cell functions and lead to more complications of cancer such as tumor invasion and metastasis (Taguchi et al., 2000). Therefore, RAGE expression may play a pivotal role in the development and progression of cancer. The linkage of RAGE/AGE has been reported to induce cellular oxidative stress and activating nuclear factor kappa B (NF-κB) signaling pathway (Yan et al., 1994; Lander et al., 1997). In humans, a high level of RAGEv1 in serum has been reported to be associated with a lower incidence of various chronic degenerative diseases, such as coronary artery disease, hypertension, diabetic complications, and rheumatoid arthritis (Emanuele et al., 2005; Falcone et al., 2005; Geroldi et al., 2005; Pullerits et al., 2005; Koyama et al., 2005; Katakami et al., 2005). Therefore, competitive inhibition of RAGE by its soluble form, RAGEv1, may attenuate the deleterious effects by decreasing expression of NF-κB and other pro-inflammatory genes in cancer cells. Hence, the objective of this study was to investigate the molecular role of RAGEv1 isoform in modulating the gene expression of NF-κB and TNF-α in human hepatocellular carcinoma HepG2 cells. This may lead to the development of an optional therapeutic target in the treatment of increasing public health threats such as liver cancer.

MATERIAL AND METHODS

Cell culture

The human cervical cancer cells (HeLa cells) and the human hepatocellular carcinoma cells (HepG2) were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum containing 100 U/mL penicillin and 100 µg/mL streptomycin. The cells were maintained at 37°C in a humidified atmosphere at 5% CO_2.

Cloning of human RAGEv1 isoform

Total cellular RNA was isolated from HeLa cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Purified mRNA was reverse-transcribed into cDNA using the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA) with oligo(dT)17 primer, following the manufacturer protocol. Resulting cDNA of all variants of RAGE was amplified with TaKaRa LA Taq DNA polymerase (Takara Bio Inc., Otsu, Shiga, Japan) using PCR primers specific to the RAGE gene (forward primer: 5'-GAAGGAAGCAGGATGGCA -3' and reverse primer: 5'-GATGGGATCTGTCTGTGGG-3'). After denaturation for 5 min at 94°C, the DNA fragments were amplified for 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2.5 min, with a final extension at 72°C for 5 min. To check their product sizes, amplified fragments of RAGE were electrophoresed on a 2% agarose gel. The PCR product with the expected fragment size of around 1200 bp was purified and cloned into the pGem-T-Easy vector system (Promega), following manufacturer instructions. Colony PCR using T7 forward primer (5'-TAATACGACTCACTATAGGG-3') and SP6 reverse primer (5'-ATTTAGGTGACACTATAGAA-3') was performed to screen colonies for expected plasmid construct (pGEM-T-Easy-RAGEv1). In addition, plasmid isolation was carried out using the QIAprep Spin Miniprep kit (Qiagen GmbH, Hilden, Germany), and subsequent restriction enzyme analysis was performed using *Hin*dIII and *Pst*I, as previously described (Hudson et al., 2008). DNA sequencing was done to verify the sequence identity. Finally, RAGEv1 was cloned into the mammalian expression vector using the pcDNA3.1/V5-His TOPO TA Expression kit (Invitrogen). Briefly, PCR amplification of the RAGEv1 fragment was carried out using pGEM-T-Easy-RAGEv1 as a PCR template. Primers used for this purpose were forward primer (5'-AGAGAATTCGAAGCAAGATGGCA-3') and reverse primer (5'-AGGTCTAGA GCAAGGCCCTCCAGTA-3'), which were designed to contain *Eco*RI and *Xba*I flanking restriction sites, respectively. Colony PCR screening was done using T7 forward primer and BGH reverse primer (5'-TAGAAGGCACAGTCGAGG-3'). The resulting plasmid construct, pcDNA3.1/V5-His TOPO TA-RAGEv1, was checked using *Bam*HI and *Eco*RV restriction enzyme digestion, and it was verified by DNA sequencing.

Transient transfection of plasmid construct containing RAGEv1 into HepG2 cells and gene expression analysis

Four million HepG2 cells were equally divided into four flasks (75 cm^2) and incubated for 48 h under 5% CO_2. The pcDNA3.1/V5-His TOPO TA-RAGEv1 construct was extracted

with the PureLink™ HiPure Plasmid Maxiprep kit (Invitrogen) and diluted to a concentration of 1 μg/μL. HepG2 cells were transfected with the plasmid construct containing RAGEv1 using lipofectamine 2000 (Invitrogen) in serum-free DMEM at a ratio of 1 μg DNA:1 μL lipofectamine, and four ratios (2 μg:2 μL, 4 μg:4 μL, 6 μg:6 μL, 8 μg:8 μL) were applied, thus reflecting an increased amount of DNA construct into the cells. For controls, non-transfected cells served as normal control cells, and transfected cells with 5 μg empty vector per 5 μL lipofectamine served as negative transfected cells. The transfection mixture was incubated with cells at 37°C under 5% CO_2 for 4 h. After the medium was changed to 10% DMEM, cells were further incubated at 37°C under 5% CO_2 for 20 h. Subsequently, RNA was extracted from cells using the QuickPrep Micro mRNA purification kit (GE Healthcare, San Diego, CA, USA) and checked for the gene expression of RAGEv1, NF-κB and TNF-α. Briefly, after reverse transcription into cDNA using ImProm-II Reverse transcriptase (Promega) and oligo (dT_{17}) primer, PCR was performed using TaKaRa LA Taq DNA polymerase (Takara Bio Inc.) and specific primers for RAGEv1 (forward primer: 5'-AGCAGTTGGAGCCTGGGTG-3' and reverse primer: 5'-GGACTCGGTAGTTGGACTTGG-3'), NF-κB (forward primer: 5'-CAGTG AGAAGGGCCGAAAGAC-3' and reverse primer: 5'-CAGGGGCAGGGAGAAGGAG-3') and TNF-α (forward primer: 5'-GGCTCCAGGCGGTGCTTGTT-3' and reverse primer: 5'-GGCTTGTCACTCGGGGTTCG-3'). β-actin transcripts, which were used as internal controls, were generated using specific primers as described elsewhere (Fisker et al., 2004).

In addition to transcripts, transfection results were also checked by detecting the RAGEv1 protein in whole-cell extracts and the secreted RAGEv1 protein in cell culture medium. In brief, HepG2 cells were transfected with the pcDNA3.1/V5-His TOPO TA-RAGEv1 construct using lipofectamine 2000 based on three different ratios of DNA:lipofectamine (1:1, 1:2, 1:3), while non-transfected HepG2 cells were negative controls. After transfection, proteins were extracted using trichloroacetic acid, separated by SDS-PAGE and detected by Western blot using anti-V5 antibody (Invitrogen), which was specific to V5 epitope of the pcDNA3.1/V5-His TOPO TA vector. Anti-mouse antibody (Cell Signaling Technology, Danvers, MA, USA) was used as the secondary antibody.

Statistical analysis

Each transfection experiment was performed in triplicate. Data are reported as means ± SD of three independent experiments. One-way ANOVA was used for analyzing the difference in gene expression between controls and transfected cells. A P value of <0.05 was considered to be significant.

RESULTS

Detection of RAGEv1 expression

We isolated RAGEv1 mRNA from HeLa cells, and DNA sequencing analysis confirmed that it was completely similar to previously identified RAGEv1 (NM_001136.3). Therefore, we made the pcDNA3.1/V5-His TOPO TA-RAGEv1 construct for transient transfection into HepG2 cell line as an *in vitro* model. Transfection studies were performed in order to evaluate both ectopic mRNA and protein levels of RAGEv1. As shown in Figure 1,

the RAGEv1 mRNA transcripts in HepG2 cells normalized to that of β-actin was found to be increased with increasing amounts of transfecting pcDNA3.1/V5-His TOPO TA-RAGEv1 construct. It reached statistically significant difference ($P < 0.05$) when 8 µg of the pcDNA3.1/V5-His TOPO TA-RAGEv1 construct was transfected into the HepG2 cells. In addition, the RAGEv1 protein was determined using Western blotting with anti-V5 antibody. Consistently, the RAGEv1 protein was highly expressed in the transfected cells and could be secreted into the culture medium of the transfected cells as shown in Figure 2.

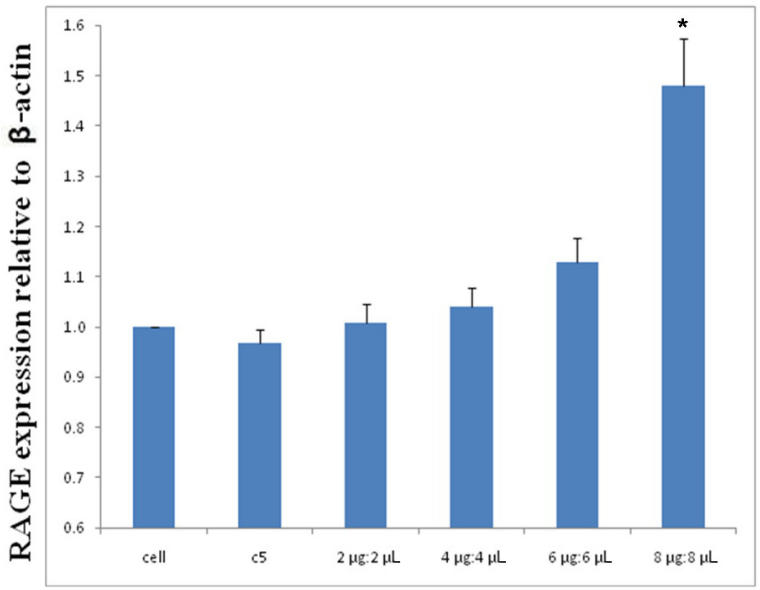

Figure 1. Expression of receptor for advanced glycation end-product variant 1 (RAGEv1) mRNA transcripts normalized to that of β-actin as detected by semi-quantitative reverse transcriptase-polymerase chain reaction in HepG2 cells transfected with increasing amounts of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct. Cell and c5 are referred to as non-transfected cells and transfected cells with 5 µg empty vector, respectively. Others show transfected cells with increasing amounts of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct from 2 to 8 µg. *Indicates a statistically significant difference ($P < 0.05$) compared to non-transfected cells.

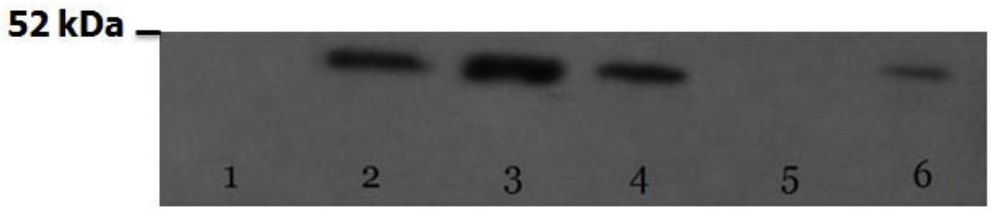

Figure 2. Detection of receptor for advanced glycation end-product variant 1 (RAGEv1) protein by Western blot using anti-V5 antibody in HepG2 cells transfected with different ratios of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct to lipofectamine. One microgram of DNA construct was used for each transfection, with increasing lipofectamine volumes of 1 to 3 µL (*lanes 2-4*). *Lanes 1*, *5* and *6* are based on detection of the RAGEv1 protein derived from non-transfected cells, culture medium from non-transfected cells and culture medium from transfected cells, respectively.

Effect of RAGEv1 expression on the expression of NF-κB and TNF-α mRNA transcripts

After different amounts of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct was transiently transfected into HepG2 cells, expression of NF-κB and TNF-α mRNA transcripts was measured relative to β-actin mRNA transcripts. As shown in Figure 3, mRNA transcripts of both genes were decreased in a concentration-dependent manner with regard to the amount of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct applied for transfection. The downregulating effect of RAGEv1 was more pronounced in case of NF-κB expression. Nevertheless, significant difference in a decreased mRNA expression of both genes was observed ($P < 0.05$) when 8 μg of the pcDNA3.1/V5-His TOPO TA-RAGEv1 construct was used for transfection.

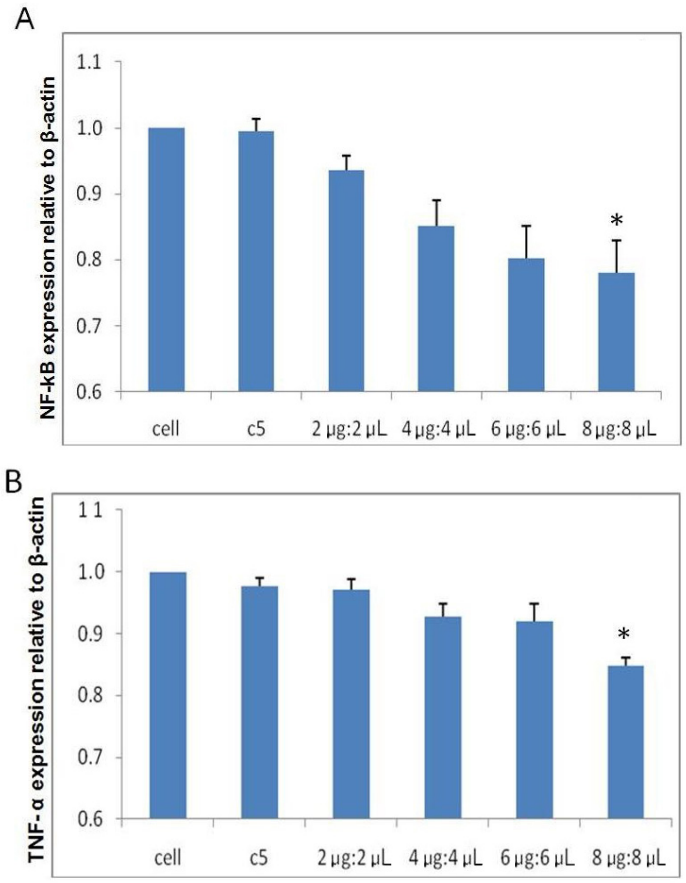

Figure 3. Expression of NF-κB (**A**) and TNF-α (**B**) mRNA transcripts normalized to that of β-actin as detected by semi-quantitative reverse transcriptase-polymerase chain reaction in HepG2 cells transfected with increasing amounts of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct. Cell and c5 are referred to as non-transfected cells and transfected cells with 5 μg empty vector, respectively. Others show transfected cells with increasing amounts of pcDNA3.1/V5-His TOPO TA-RAGEv1 construct from 2 to 8 μg. *Indicates a statistically significant difference ($P < 0.05$) compared to non-transfected cells.

DISCUSSION

RAGEv1 is one of the most abundant alternative splice variants of the RAGE gene, second to the full-length form (Hudson et al., 2008). For the direct identification of RAGEv1 mRNA in this study, nucleotide sequencing was performed and confirmed. In this study, we detected the expressed RAGEv1 protein using anti-V5 antibody against the V5 epitope, since the RAGEv1 cDNA was cloned into the mammalian vector containing the V5 tag. According to our cloning approach, the reverse primer was designed to be particularly mutated, thus destroying the stop codon and reading through the target tag.

From previous studies, we already knew that RAGE expression could be regulated by the NF-κB signaling pathway (Ahmed, 2005). According to the promoter analysis, there are two NF-κB binding sites on RAGE promoter, thus supporting the idea that upregulation of NF-κB leads to an enhanced expression of the RAGE gene and vice versa (Rojas et al., 2010). Since more than two hundred genes may be regulated by NF-κB transcription factor, including anti-apoptotic, cellular transformation inducer, metastatic, chemo-resistant, and radio-resistant genes, activation of these genes may result in the aggressiveness of cancer progression. NF-κB also activates the expression of cyclin D1 and apoptotic suppressor protein, such as Bcl-2 and Bcl-XL, which are involved in metastasis and angiogenesis (Aggarwal and Shishodia, 2006). Furthermore, it has been demonstrated that upregulation of the RAGE gene could promote cancer invasiveness (Hirata et al., 2003). Taken together, the inhibition of RAGE gene expression could be an alternative way for cancer treatment. Recent study showed that RAGEv1 expression inhibited tumor growth *in vitro* and *in vivo* by reduced activation of JNK signaling pathway (Kalea et al., 2010). Based on the quantitative analysis of RAGE mRNA expression in hepatocellular carcinoma patients (HCC), it has been revealed that HCC patients have higher RAGE expression compared to non-HCC ($P < 0.01$). Moreover, HCC patients showed a significant increase in RAGE expression as compared to patients with non-cancerous hepatic lesions (cirrhosis; $P < 0.01$) (Hiwatashi et al., 2008). This is evidence of a firmly established association between HCC and expression of RAGE, thus emphasizing the devastating role of the RAGE gene in pathogenesis. It is worth noting that Kalea et al. (2010) have demonstrated that RAGEv1 is expressed at a low level in cancer tissues. The development of cancer may be possibly due to the insufficient concentration of such a protective biomarker as RAGEv1 in the body.

After binding with ligands, the RAGE-ligand complex induces an intracellular signal, and this type of activation normally leads to an increase in oxidative stresses (Lander et al., 1997). In addition, the RAGE-ligand interaction promotes the expression of several biomarkers such as IL-1α, IL-6 and TNF-α (Neumann et al., 1999). If the binding of ligand and RAGE is impeded by competitive inhibitors such as RAGEv1, the signal activation should be decreased. In this study, we found that higher expressed levels of RAGEv1 based on increasing amounts of plasmid construct containing RAGEv1 for transfection resulted in a decreased expression of NF-κB and TNF-α in a concentration-dependent manner. In particular, the results demonstrated that both NF-κB and TNF-α mRNA transcripts significantly decreased after transfection of 8 μg RAGEv1 recombinant plasmid ($P < 0.05$), whereas the rest (2, 4 and 6 μg of the construct) did not give any significant result. We did not transfect HepG2 cells using higher concentrations of plasmid construct because cell viability dropped to less than 50% compared to non-transfected cells (data not shown).

This study shows a molecular role of RAGEv1 in suppressing gene expression of NF-κB and TNF-α genes in human liver cancer cells. This finding is of great interest since RAGEv1 may serve as a prime candidate target for developing anti-cancer drugs in the future, especially for HCC. Like full-length RAGE, RAGEv1 is a native plasma soluble protein in the human bloodstream, and thus, the nuisance effects of RAGEv1-based therapy should be notably less than foreign molecules. In addition, it can be therapeutically applied to other chronic degenerative diseases, known to be pathologically associated with RAGE, such as cardiovascular disease (Falcone et al., 2005), diabetes (Rojas and Morales, 2004; Katakami et al., 2005), hypertension (Geroldi et al., 2005), and Alzheimer's disease (Emanuele et al., 2005).

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REFERENCES

Aggarwal BB and Shishodia S (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol.* 71: 1397-1421.

Ahmed N (2005). Advanced glycation endproducts-role in pathology of diabetic complications. *Diabetes Res. Clin. Pract.* 67: 3-21.

Emanuele E, D'Angelo A, Tomaino C, Binetti G, et al. (2005). Circulating levels of soluble receptor for advanced glycation end products in Alzheimer disease and vascular dementia. *Arch. Neurol.* 62: 1734-1736.

Falcone C, Emanuele E, D'Angelo A, Buzzi MP, et al. (2005). Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men. *Arterioscler. Thromb. Vasc. Biol.* 25: 1032-1037.

Fernandez-Botran R, Crespo FA and Sun X (2002). Soluble cytokine receptors in biological therapy. *Expert. Opin. Biol. Ther.* 2: 585-605.

Fisker S, Hansen B, Fuglsang J, Kristensen K, et al. (2004). Gene expression of the GH receptor in subcutaneous and intraabdominal fat in healthy females: relationship to GH-binding protein. *Eur. J. Endocrinol.* 150: 773-777.

Geroldi D, Falcone C, Emanuele E, D'Angelo A, et al. (2005). Decreased plasma levels of soluble receptor for advanced glycation end-products in patients with essential hypertension. *J. Hypertens.* 23: 1725-1729.

Hirata K, Takada M, Suzuki Y and Kuroda Y (2003). Expression of receptor for advanced glycation end products (RAGE) in human biliary cancer cells. *Hepatogastroenterology* 50: 1205-1207.

Hiwatashi K, Ueno S, Abeyama K, Kubo F, et al. (2008). A novel function of the receptor for advanced glycation end-products (RAGE) in association with tumorigenesis and tumor differentiation of HCC. *Ann. Surg. Oncol.* 15: 923-933.

Hofmann MA, Drury S, Fu C, Qu W, et al. (1999). RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97: 889-901.

Hori O, Brett J, Slattery T, Cao R, et al. (1995). The receptor for advanced glycation end products (RAGE) is a cellular binding site for amphoterin. Mediation of neurite outgrowth and co-expression of rage and amphoterin in the developing nervous system. *J. Biol. Chem.* 270: 25752-25761.

Hudson BI, Carter AM, Harja E, Kalea AZ, et al. (2008). Identification, classification, and expression of RAGE gene splice variants. *FASEB J.* 22: 1572-1580.

Kalea AZ, See F, Harja E, Arriero M, et al. (2010). Alternatively spliced RAGEv1 inhibits tumorigenesis through suppression of JNK signaling. *Cancer Res.* 70: 5628-5638.

Katakami N, Matsuhisa M, Kaneto H, Matsuoka TA, et al. (2005). Decreased endogenous secretory advanced glycation end product receptor in type 1 diabetic patients: its possible association with diabetic vascular complications. *Diabetes Care* 28: 2716-2721.

Koyama H, Shoji T, Yokoyama H, Motoyama K, et al. (2005). Plasma level of endogenous secretory RAGE is associated with components of the metabolic syndrome and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 25: 2587-2593.

Lander HM, Tauras JM, Ogiste JS, Hori O, et al. (1997). Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. *J. Biol. Chem.* 272: 17810-17814.

Neeper M, Schmidt AM, Brett J, Yan SD, et al. (1992). Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J. Biol. Chem.* 267: 14998-15004.

Neumann A, Schinzel R, Palm D, Riederer P, et al. (1999). High molecular weight hyaluronic acid inhibits advanced glycation endproduct-induced NF-kappaB activation and cytokine expression. *FEBS Lett.* 453: 283-287.

Pullerits R, Bokarewa M, Dahlberg L and Tarkowski A (2005). Decreased levels of soluble receptor for advanced glycation end products in patients with rheumatoid arthritis indicating deficient inflammatory control. *Arthritis Res. Ther.* 7: R817-R824.

Rojas A and Morales MA (2004). Advanced glycation and endothelial functions: a link towards vascular complications in diabetes. *Life Sci.* 76: 715-730.

Rojas A, Figueroa H and Morales E (2010). Fueling inflammation at tumor microenvironment: the role of multiligand/RAGE axis. *Carcinogenesis* 31: 334-341.

Schmidt AM, Yan SD, Yan SF and Stern DM (2001). The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J. Clin. Invest.* 108: 949-955.

Taguchi A, Blood DC, del TG, Canet A, et al. (2000). Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. *Nature* 405: 354-360.

Yan SD, Schmidt AM, Anderson GM, Zhang J, et al. (1994). Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. *J. Biol. Chem.* 269: 9889-9897.

Yonekura H, Yamamoto Y, Sakurai S, Petrova RG, et al. (2003). Novel splice variants of the receptor for advanced glycation end-products expressed in human vascular endothelial cells and pericytes, and their putative roles in diabetes-induced vascular injury. *Biochem. J.* 370: 1097-1109.