J. Cell Commun. Signal. (2013) 7:19–23
DOI 10.1007/s12079-012-0181-3

NUTS AND BOLTS

# A multicellular signal transduction network of AGE/RAGE signaling

**Sowmya Soman · Rajesh Raju · Varot K. Sandhya · Jayshree Advani · Aafaque Ahmad Khan · H. C. Harsha · T. S. Keshava Prasad · P. R. Sudhakaran · Akhilesh Pandey · Puneeth K. Adishesha**

Received: 22 October 2012 / Accepted: 31 October 2012 / Published online: 19 November 2012


**Abbreviations**

| | |
|---|---|
| RAGE | Receptor for advanced glycation end products |
| HMGB1 | High mobility group box protein 1 |
| sRAGE | Soluble RAGE |
| CML | N(6)(carboxymethyl)lysine |
| CEL | N(6)(carboxyethyl)lysine |
| MMPs | Matrix metalloproteinases |
| PTMs | Post-translational modifications |
| PPIs | Protein-protein interactions |
| BioPAX | Biological PAthway eXchange |
| SBML | Systems Biology Markup Language |
| PSI-MI | Proteomics Standards Initiative for Molecular Interaction |

## Introduction

Advanced glycation end products (AGEs) are heterogeneous glycated products of proteins, lipids and nucleotides. The major receptor for AGEs, known as receptor for

Sowmya Soman and Rajesh Raju contributed equally

S. Soman · R. Raju · V. K. Sandhya · J. Advani · A. A. Khan · H. C. Harsha · T. S. K. Prasad · P. K. Adishesha (✉)
Institute of Bioinformatics, International Technology Park, Bangalore 560066, India
e-mail: puneeth@ibioinformatics.org

S. Soman
e-mail: sowmya@ibioinformatics.org

R. Raju
e-mail: rajesh@ibioinformatics.org

V. K. Sandhya
e-mail: sandhya@ibioinformatics.org

J. Advani
e-mail: jayshree@ibioinformatics.org

A. A. Khan
e-mail: aafaque@ibioinformatics.org

H. C. Harsha
e-mail: harsha@ibioinformatics.org

T. S. K. Prasad
e-mail: keshav@ibioinformatics.org

P. R. Sudhakaran
Department of Computational Biology and Bioinformatics, State Inter-University Centre of Excellence in Bioinformatics, University of Kerala, Thiruvananthapuram 695581, India
e-mail: prslab@gmail.com

A. Pandey
McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore 21205 MD, USA
e-mail: pandey@jhmi.edu

A. Pandey
Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore 21205 MD, USA

A. Pandey
Department of Oncology, Johns Hopkins University School of Medicine, Baltimore 21205 MD, USA

A. Pandey
Department of Pathology, Johns Hopkins University School of Medicine, Baltimore 21205 MD, USA

advanced glycation end products (RAGE or AGER), is a multi-ligand transmembrane receptor of immunoglobulin superfamily. It has an extracellular region, a transmembrane domain and a short cytoplasmic domain. Extracellular region of RAGE consists of one V type (critical for ligand binding) and two C type immunoglobulin domains (Schmidt et al. 1994a, b). Although the short cytoplasmic tail of 43 amino acid residues is found to be important for the signaling events mediated by RAGE, it does not have any known domain or motif (Neeper et al. 1992). The other cell surface receptors for AGEs include dolichyl-diphosphooligosaccharide-protein glycosyltransferase (AGE-R1) (Li et al. 1996), protein kinase C substrate, 80KH phosphoprotein (AGE-R2) (Goh et al. 1996), galectin-3 (AGE-R3) (Vlassara et al. 1995), and class A macrophage scavenger receptors type I and II. RAGE is also considered as a pattern recognition receptor due to its ability to bind different AGEs. RAGE has numerous extracellular ligands in addition to AGEs, which include extracellular high mobility group box-1 (HMGB1), S100 family of calcium binding proteins and amyloid-beta peptide (Fritz 2011).

RAGE is expressed in diverse tissues such as lung, heart, kidney, brain, and skeletal muscle and in a variety of cells including endothelial cells, macrophages/monocytes, neutrophils, and lymphocytes (Brett et al. 1993; Ding and Keller 2005; Neeper et al. 1992). RAGE has been implicated in the pathogenesis of diverse diseases such as diabetes, cardiovascular disorders, arthritis, cancers and neurological disorders (Yan et al. 2009). Interactions of AGEs with their receptors alter cell function through the generation of free radicals (Schmidt et al. 1994a, b). In diabetes, interaction of AGEs with RAGE induces oxidative stress and inflammatory reactions thereby resulting in vascular damage and related complications (Yamagishi 2011). RAGE also plays an important role in the progression of atherosclerosis through oxidative stress and proinflammatory responses (Sun et al. 2009). Expression of RAGE in synovial tissue, T cells, B cells and macrophages of arthritic patients implies its significance in inflammatory joint disorders (Drinda et al. 2005). Overexpression of RAGE has also been reported in various types of cancers such as pancreatic, gastric, breast, lung cancers and lymphoma (Logsdon et al. 2007). Knockdown of RAGE expression was shown to inhibit ductal neoplasia in an animal model of pancreatic cancer (DiNorcia et al. 2012). A recent study by Liang et al. reported that the inactivation of RAGE in colorectal cancer cells reduced angiogenesis (Liang et al. 2011).

The signaling events mediated by RAGE are complex due to the diversity of its ligands and their diverse effects mediated in different cell types. AGE/RAGE signaling in endothelial cells is reported to modulate oxidative stress, inflammation, apoptosis, autophagy, endothelial-mesenchymal-transition, endothelial permeability and dysfunction (Toma et al. 2009; Xu et al. 2010; Li et al. 2011; Xie et al. 2011; Ma et al. 2010; Del Turco et al. 2011). In smooth muscle cells, AGE/RAGE interaction leads to generation of reactive oxygen species, autophagy, proliferation and calcification (Yoon et al. 2009; Hu et al. 2012; Yuan et al. 2011; Tanikawa et al. 2009). AGE/RAGE signaling is reported to mediate proliferation in lymphocytes (Takahashi et al. 2010). In fibroblasts, it induces migration, inflammation and apoptosis (Liu et al. 2010; Shimoda et al. 2011). A diverse array of molecules and signaling modules were identified to be activated by RAGE depending on the intensity and duration of RAGE ligation. Specific signaling modules such as ERK1/2 (Lander et al. 1997), p38 MAPK (Lander et al. 1997), CDC42/RAC (Bondeva et al. 2011), SAPK/JNK (Hu et al. 2012) and NF-κB (Liu et al. 2010) have been shown to be triggered by AGE/RAGE interaction in different cell types.

Currently, there are no resources, which contain RAGE signaling pathway data for visualization and analysis. Therefore, we have gathered signaling pathway reactions induced by AGE/RAGE interaction in diverse cell types and tissues from literature. We have also cataloged genes transcriptionally regulated by AGE/RAGE system in humans along with their transcriptional regulators. We have provided the AGE/RAGE signaling pathway data to scientific community through NetPath (http://www.netpath.org), a resource of signaling pathways developed by us (Kandasamy et al. 2010).

## Methodology

A PubMed search using the search terms ‘advanced glycation end products’, ‘receptor for advanced glycation end-products’ and its gene aliases were carried out. The signaling pathway information interpreted from these published research articles were recorded into a web-based tool called ‘PathBuilder’ (Kandasamy et al. 2009). AGE/RAGE signaling ex-vivo is mostly studied using the AGEs such as AGE-modified albumin, N(6)(carboxymethyl)lysine (CML), N(6)(carboxyethyl)-lysine (CEL) and pentosidine among many others. In order to ascertain specificity to AGE/RAGE signaling, we have considered pathway reactions only if they were also proved to be affected when RAGE was inhibited by different approaches. Thus, reactions induced by AGEs but are proved to be independent of RAGE are not considered. The categorization of individual molecular reactions involved in RAGE signaling into protein-protein interactions, enzyme-substrate reactions, activation-inhibition events, protein translocation events, and gene regulatory events were based on the criteria as described before (Nanjappa et al. 2011; Raju et al. 2011a, b; Telikicherla et al. 2011; Goel et al. 2011a, b). The reactions were further categorized with respect to specific cell types in which they are studied. We have also captured post-translational modifications (PTMs) identified to mediate individual reactions in AGE/RAGE signaling. These PTMs were mapped to their specific or longest protein isoform in RefSeq. We applied a set of criteria to filter high-confidence reactions of AGE/

RAGE signaling (Raju et al. 2011a, b). The reactions filtered using these criteria were used to represent topology-devised graphical map of the signaling network. This map was manually drawn using the visualization tool named 'PathVisio' (http://www.PathVisio.org).

## Results and discussion

We have screened over 6,000 research articles to identify molecules and their reactions reported to be induced by AGE/RAGE system and documented 95 molecules involved in the AGE/RAGE signaling. These molecules formed the components of 14 PPIs, 55 catalysis (2 direct and 53 induced), 19 protein translocation and 27 spatiotemporal activation/inhibition events. In all, 60 genes regulated by AGE/RAGE signaling reported in different types of cells in humans were also cataloged. Further, 6 transcriptional regulators (transcription factors, co-activators or co-repressors) reported to modulate the expression of these genes by RAGE signaling were also identified. An overview of the AGE/RAGE signaling pathway data is available through NetPath (http://www.netpath.org/pathways?path_id=NetPath_137). A graphical map of reactions involved in AGE/RAGE signaling filtered using NetSlim criteria is represented in Fig. 1.

The cellular processes induced by AGE/RAGE system in specific cells or cell types are provided in the map. The current study also highlights the molecules such as AKT1 and ezrin (EZR), which are differentially regulated by RAGE signaling in different cell types. The map is made dynamic with individual reactions linked to their corresponding PubMed identifiers and with each of the proteins linked to respective molecule pages in NetPath. The molecule pages in NetPath are also linked to external repositories such as HPRD (Goel et al. 2011a, b),

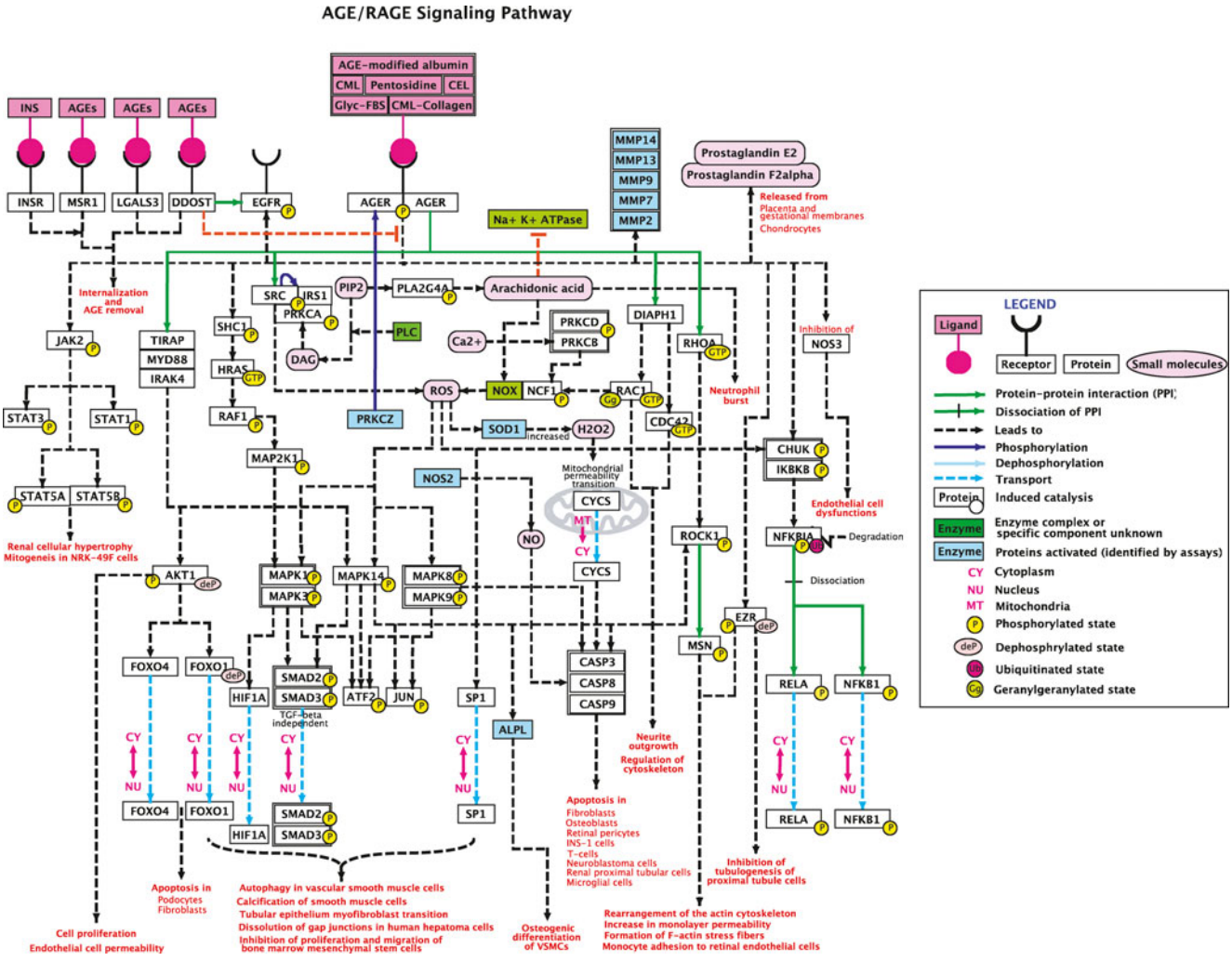


**Fig. 1** Schematic representation of AGE/RAGE signaling pathway in NetSlim- This map represents the NetSlim reactions of AGE/RAGE signaling pathway in different cell types. The different types of reactions are distinguished by colors described in the legend. The map with reactions linked to their corresponding PubMed identifiers is available at http://www.netpath.org/netslim/age_signaling_pathways.html

Entrez gene (Maglott et al. 2005), OMIM (Hamosh et al. 2005) and Swiss-Prot (Boeckmann et al. 2003) for ease of navigation to more information on each of the proteins. This map with citation is freely available in NetSlim at (http://www.netpath.org/netslim/age_signaling_pathways.html).

## Review of AGE/RAGE signaling pathway data

AGE/RAGE signaling pathway data after internal review were uploaded into NetPath to facilitate review of the pathway data by a pathway authority, an expert in this signaling pathway (PRS, a co-author in this manuscript). We have incorporated the suggestions of the pathway authority to accommodate broad opinion on AGE/RAGE signaling in the scientific community.

## Data availability

The data obtained in this study are freely available from NetPath and NetSlim resources under an adaptive creative commons license (http://www.creativecommons.org/licenses/by-nc/2.5) for unrestricted non-commercial use. The AGE/RAGE signaling pathway data is available for download in standard data exchange formats such as Proteomics Standards Initiative for Molecular Interaction (PSI-MI version 2.5) (Hermjakob et al. 2004), Biological PAthway eXchange (BioPAX level 3) (Demir et al. 2010) and Systems Biology Markup Language (SBML 2.1) (Hucka et al. 2003). The pathway maps are available in GPML, GenMAPP and PDF formats for use by both academicians and researchers. We also submitted the AGE/RAGE signaling map to WikiPathways (http://www.wikipathways.org/index.php/Pathway:WP2324). The list of genes identified to be regulated by AGE/RAGE system in humans is available for download in excel and tab-delimited formats. AGE/RAGE signaling pathway in NetPath and NetSlim will be periodically updated as more information becomes available.

## Conclusions

As RAGE signaling is being associated with several human diseases such as diabetes, cardiovascular diseases, cancer and neurological disorders, it is gaining importance in the recent times. Therapeutic targeting of multi-ligand receptors such as RAGE poses tremendous challenge to the scientific community due to the differences in cellular processes induced by their multiple ligands in diverse cell types. However, we report that even within a ligand group (AGEs), there are differences in signaling as exemplified by both activation and inhibition of molecules such as AKT and EZR in different cell types. We believe that further studies to elucidate cell type specific effects of AGEs will reveal many such molecules that are differentially regulated in different cell types. Together, our effort will serve as a platform for visualization and analysis of signaling data relevant to RAGE signaling derived from different high-throughput technologies in future.

**Acknowledgments** We thank the Department of Biotechnology (DBT), Government of India for research support to the Institute of Bioinformatics, Bangalore. Varot K. Sandhya is a recipient of INSPIRE Fellowship from the Department of Science and Technology, Government of India. H. C. Harsha is a Wellcome Trust-DBT India Alliance Early Career Fellow.

**Conflict of interests** The author(s) declared no conflicts of interests.

## References

Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M (2003) The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nucleic Acids Res 31:365–370

Bondeva T, Wojciech S, Wolf G (2011) Advanced glycation end products inhibit adhesion ability of differentiated podocytes in a neuropilin-1-dependent manner. Am J Physiol Renal Physiol 301:F852–F870

Brett J, Schmidt AM, Yan SD, Zou YS, Weidman E, Pinsky D, Nowygrod R, Neeper M, Przysiecki C, Shaw A et al (1993) Survey of the distribution of a newly characterized receptor for advanced glycation end products in tissues. Am J Pathol 143:1699–1712

Del Turco S, Navarra T, Gastaldelli A, Basta G (2011) Protective role of adiponectin on endothelial dysfunction induced by AGEs: a clinical and experimental approach. Microvasc Res 82:73–76

Demir E, Cary MP, Paley S, Fukuda K, Lemer C, Vastrik I, Wu G et al (2010) The BioPAX community standard for pathway data sharing. Nat Biotechnol 28:935–942

Ding Q, Keller JN (2005) Splice variants of the receptor for advanced glycosylation end products (RAGE) in human brain. Neurosci Lett 373:67–72

DiNorcia J, Lee MK, Moroziewicz DN, Winner M, Suman P, Bao F, Remotti HE, Zou YS, Yan SF, Qiu W, Su GH, Schmidt AM, Allendorf JD (2012) RAGE gene deletion inhibits the development and progression of ductal neoplasia and prolongs survival in a murine model of pancreatic cancer. J Gastrointest Surg 16:104–112, discussion 112

Drinda S, Franke S, Ruster M, Petrow P, Pullig O, Stein G, Hein G (2005) Identification of the receptor for advanced glycation end products in synovial tissue of patients with rheumatoid arthritis. Rheumatol Int 25:411–413

Fritz G (2011) RAGE: a single receptor fits multiple ligands. Trends Biochem Sci 36:625–632

Goel R, Muthusamy B, Pandey A, Prasad TSK (2011a) Human protein reference database and human proteinpedia as discovery resources for molecular biotechnology. Mol Biotechnol 48:87–95

Goel R, Raju R, Maharudraiah J, Kumar GSS, Ghosh K, Kumar A, Lashmi PT et al (2011b) A signaling network of thyroid-stimulating hormone. J Proteonomics Bioinformatics 4:238–241

Goh KC, Lim YP, Ong SH, Siak CB, Cao X, Tan YH, Guy GR (1996) Identification of p90, a prominent tyrosine-phosphorylated

protein in fibroblast growth factor-stimulated cells, as 80K-H. J Biol Chem 271:5832–5838

Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA (2005) Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 33:D514–D517

Hermjakob H, Montecchi-Palazzi L, Bader G, Wojcik J, Salwinski L, Ceol A, Moore S et al (2004) The HUPO PSI's molecular interaction format–a community standard for the representation of protein interaction data. Nat Biotechnol 22:177–183

Hu P, Lai D, Lu P, Gao J, He H (2012) ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells. Int J Mol Med 29:613–618

Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, Arkin AP et al (2003) The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 19:524–531

Kandasamy K, Keerthikumar S, Raju R, Prasad TSK, Ramachandra YL, Mohan S, Pandey A (2009) PathBuilder–open source software for annotating and developing pathway resources. Bioinformatics 25:2860–2862

Kandasamy K, Mohan SS, Raju R, Keerthikumar S, Kumar GS, Venugopal AK, Telikicherla D et al (2010) NetPath: a public resource of curated signal transduction pathways. Genome Biol 11:R3

Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM (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

Li YM, Mitsuhashi T, Wojciechowicz D, Shimizu N, Li J, Stitt A, He C, Banerjee D, Vlassara H (1996) Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins. Proc Natl Acad Sci U S A 93:11047–11052

Li BY, Li XL, Gao HQ, Zhang JH, Cai Q, Cheng M, Lu M (2011) Grape seed procyanidin B2 inhibits advanced glycation end product-induced endothelial cell apoptosis through regulating GSK3beta phosphorylation. Cell Biol Int 35:663–669

Liang H, Zhong Y, Zhou S, Peng L (2011) Knockdown of RAGE expression inhibits colorectal cancer cell invasion and suppresses angiogenesis in vitro and in vivo. Cancer Lett 313:91–98

Liu Y, Liang C, Liu X, Liao B, Pan X, Ren Y, Fan M, Li M, He Z, Wu J, Wu Z (2010) AGEs increased migration and inflammatory responses of adventitial fibroblasts via RAGE, MAPK and NF-kappaB pathways. Atherosclerosis 208:34–42

Logsdon CD, Fuentes MK, Huang EH, Arumugam T (2007) RAGE and RAGE ligands in cancer. Curr Mol Med 7:777–789

Ma J, Liu T, Dong X (2010) Advanced glycation end products of bovine serum albumin-induced endothelial-to-mesenchymal transition in cultured human and monkey endothelial cells via protein kinase B signaling cascades. Mol Vis 16:2669–2679

Maglott D, Ostell J, Pruitt KD, Tatusova T (2005) Entrez Gene: gene-centered information at NCBI. Nucleic Acids Res 33:D54–D58

Nanjappa V, Raju R, Muthusamy B, Sharma J, Thomas K, Nidhina PAH, Harsha HC, Pandey A, Anilkumar G, Prasad TSK (2011) A comprehensive curated reaction map of leptin signaling pathway. J Proteonomics Bioinformatics 4:184–189

Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, Elliston K, Stern D, Shaw A (1992) Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. J Biol Chem 267:14998–15004

Raju R, Balakrishnan L, Nanjappa V, Bhattacharjee M, Getnet D, Muthusamy B, Kurian Thomas J, et al. (2011) A comprehensive manually curated reaction map of RANKL/RANK-signaling pathway. Database (Oxford) 2011: bar021

Raju R, Nanjappa V, Balakrishnan L, Radhakrishnan A, Thomas JK, Sharma J, Tian M, et al., (2011) NetSlim: high-confidence curated signaling maps. Database (Oxford) 2011: bar032

Schmidt AM, Hasu M, Popov D, Zhang JH, Chen J, Yan SD, Brett J, Cao R, Kuwabara K, Costache G et al (1994a) 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

Schmidt AM, Hori O, Brett J, Yan SD, Wautier JL, Stern D (1994b) Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb 14:1521–1528

Shimoda H, Nakamura S, Morioka M, Tanaka J, Matsuda H, Yoshikawa M (2011) Effect of Cinnamoyl and Flavonol Glucosides Derived from Cherry Blossom Flowers on the Production of Advanced Glycation End Products (AGEs) and AGE-induced Fibroblast Apoptosis. Phytother Res

Sun L, Ishida T, Yasuda T, Kojima Y, Honjo T, Yamamoto Y, Yamamoto H, Ishibashi S, Hirata K, Hayashi Y (2009) RAGE mediates oxidized LDL-induced pro-inflammatory effects and atherosclerosis in non-diabetic LDL receptor-deficient mice. Cardiovasc Res 82:371–381

Takahashi HK, Zhang J, Mori S, Liu K, Wake H, Liu R, Sadamori H, Matsuda H, Yagi T, Yoshino T, Nishibori M (2010) Prostaglandin E2 inhibits advanced glycation end product-induced adhesion molecule expression on monocytes, cytokine production, and lymphocyte proliferation during human mixed lymphocyte reaction. J Pharmacol Exp Ther 334:964–972

Tanikawa T, Okada Y, Tanikawa R, Tanaka Y (2009) Advanced glycation end products induce calcification of vascular smooth muscle cells through RAGE/p38 MAPK. J Vasc Res 46:572–580

Telikicherla D, Ambekar A, Palapetta SM, Dwivedi SB, Raju R, Sharma J, Prasad TSK, Ramachandra Y, Mohan SS, Maharudraiah J, Mukherjee S, Pandey A (2011) A comprehensive curated resource for follicle stimulating hormone signaling. BMC Res Notes 4:408

Toma L, Stancu CS, Botez GM, Sima AV, Simionescu M (2009) Irreversibly glycated LDL induce oxidative and inflammatory state in human endothelial cells; added effect of high glucose. Biochem Biophys Res Commun 390:877–882

Vlassara H, Li YM, Imani F, Wojciechowicz D, Yang Z, Liu FT, Cerami A (1995) Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex. Mol Med 1:634–646

Xie Y, You SJ, Zhang YL, Han Q, Cao YJ, Xu XS, Yang YP, Li J, Liu CF (2011) Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells. Mol Med Report 4:459–464

Xu Y, Wang S, Feng L, Zhu Q, Xiang P, He B (2010) Blockade of PKC-beta protects HUVEC from advanced glycation end products induced inflammation. Int Immunopharmacol 10:1552–1559

Yamagishi S (2011) Role of advanced glycation end products (AGEs) and receptor for AGEs (RAGE) in vascular damage in diabetes. Exp Gerontol 46:217–224

Yan SF, Ramasamy R, Schmidt AM (2009) Receptor for AGE (RAGE) and its ligands-cast into leading roles in diabetes and the inflammatory response. J Mol Med (Berl) 87:235–247

Yoon SJ, Yoon YW, Lee BK, Kwon HM, Hwang KC, Kim M, Chang W, Hong BK, Lee YH, Park SJ, Min PK, Rim SJ (2009) Potential role of HMG CoA reductase inhibitor on oxidative stress induced by advanced glycation endproducts in vascular smooth muscle cells of diabetic vasculopathy. Exp Mol Med 41:802–811

Yuan X, Zhang Z, Gong K, Zhao P, Qin J, Liu N (2011) Inhibition of reactive oxygen species/extracellular signal-regulated kinases pathway by pioglitazone attenuates advanced glycation end products-induced proliferation of vascular smooth muscle cells in rats. Biol Pharm Bull 34:618–623