A multicellular signal transduction network of AGE/RAGE signaling

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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 glycation products of proteins, lipids and nucleotides. The major receptor for AGEs, known as receptor for

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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- κ B (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 Net-Path (<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.

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