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Research article

Lower expression level of two RAGE alternative splicing isoforms in Alzheimer's disease

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HIGHLIGHTS

- RAGE is a cell surface receptor involved in neurodegeneration.
- RAGE has multiple alternative splicing isoforms in human cell.
- Two of RAGE alternative splicing isoforms expressed lower in Alzheimer's disease compared with control.
- These isoforms may involve in the development of Alzheimer's disease.

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ABSTRACT

Alternative splicing (AS) is a common phenomenon in gene expression of eukaryotic organisms, especially in mammals, producing multiple gene isoforms from a single gene that involve in physiological and pathological processes. Receptor for advanced glycation end products (RAGE) has multiple AS isoforms with significant tissue and organism specificity. RAGE signaling has been reported involved in the onset and development of Alzheimer's disease (AD) and the roles of RAGE AS isoforms have not yet been fully illustrated in AD pathogenesis. In the present study, two of RAGE AS isoforms (RAGEΔ and sRAGEΔ) were investigated in the human brain specimens from AD and age-matched control subjects. The expression of these isoforms was found brain-region specific, and significant lower expression levels of both RAGEΔ and sRAGEΔ were detected in multiple brain regions of AD subjects than control subjects. Data indicated tight association between the AS isoforms (RAGEΔ and sRAGEΔ) and neurodegeneration. An antagonistic pairing model has been suggested to explain the working mechanism of AS isoforms on gene regulation and pathological development.

1. Introduction

Alternative splicing (AS) occurs in the majority of human genes with high tissue specificity and is an effective way to increase protein diversity [1,2]. The literature indicated that AS is involved in genomic evolution, physiological development and pathogenesis [3–5]. For example, the AS isoform Stat3β contributed to constitutive Stat3 activation in oncogenesis [6], and the AS isoforms could increase the sensitivity of G-protein dependent calcium channels which are involved in pain control [7]. The receptor for advanced glycation end products (RAGE) is a member of immunoglobulin (Ig) superfamily, normally located on cytoplasm membrane, has multiple AS isoforms [8]. This receptor binds to multiple ligands including β-amyloid (Aβ), calgranulin, high-mobility group box-1 (HMGB1) and DNA fragments to trigger immune response and inflammatory signaling pathways that associate with neurodegeneration, diabetic pathogenesis and vascular disorders [9–11]. It is well known that Aβ plays a pivotal role in the pathogenesis of Alzheimer's disease (AD), and numerous evidence has indicated that RAGE signaling took part in the onset and development of AD [12,13]. For example, blockage of RAGE signaling could reduce cerebral Aβ accumulation and improve learning and memory in APP/PS1 mice [13]. Recent studies indicate that RAGE has varied AS isoforms in different tissues and different organisms, with 12 isoforms detected in the human brain, 16 isoforms in the human kidney and 15 isoforms in the human pancreas. Meanwhile, the isoforms detected in other organisms such as rat, mouse, monkey, cattle and pig had varied structures and less number of isoforms [14]. Our previous study also showed that RAGE AS isoforms are

Abbreviation: RAGE, receptor for advanced glycation end products; AS, alternative splicing; AD, Alzheimer's disease; IP, inferior parietal lobule; SMTG, superior middle temporal gyrus; hippo, hippocampal gyrus; CE, cerebellum.

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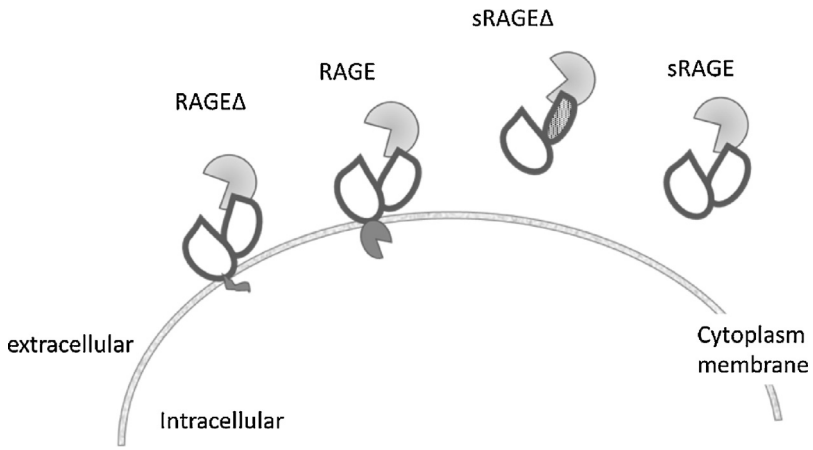

Fig. 1. Schematic representation of RAGEΔ, and sRAGEΔ compared with full length RAGE and secretory RAGE (sRAGE). RAGEΔ and full length RAGE are located on the cytoplasm membrane, while sRAGEΔ and sRAGE secreted out of the cytoplasm membrane. The RAGEΔ has altered intracellular interacting domain, and the sRAGEΔ has transmembrane and intracellular domains removed with an altered immunoglobulin domain (C2, dotted).

highly tissue specific and 6 novel AS isoforms had been cloned from hippocampus region of the human brain [15]. In this study two novel AS isoforms of human RAGE were further investigated to reveal their correlation with AD pathogenesis.

2. Material and methods

Studies have been conducted in multiple human brain regions (inferior parietal lobule (IP), superior middle temporal gyrus (SMTG), hippocampal gyrus (hippo), and cerebellum (CE)). The specimens were obtained from the tissue bank in Sanders–Brown Center on Aging, University of Kentucky. The basic information about the subjects was reported previously [16], with eight AD subjects (two males and six females, average age 85.75 ± 5.73 years old), and eight control subjects (three males and five females, average age 85.38 ± 5.80 years old). All these samples were harvested shortly after death (average PMI for control is 2.97 ± 0.71 h, and 3.07 ± 0.94h for AD subjects) and stored at $-80\,^{\circ}$C until usage.

The expression levels of the isoforms were measured following the established RT-PCR procedure [15], the primers used for RAGEΔ were: 5′-AGC CGT GCT GTC AGC ATC AGC ATC-3′, and 5′-ATT CAG TTC TGC TCG GCG TTG CCG-3′. The primers used for sRAGEΔ were: 5′-GGG AAG CCC CTG ACC AGG AGA CAC-3′, and 5′-TCC CAC AGA GCC TCA CAT GTG TTG-3′. cDNA was produced by Reverse Transcription System from Promega (A3500, Madison, WI). Thermocycle parameters are: 95 °C for 45 s, 58 °C for 40 s, 72 °C for 55 s in the first five cycles and then followed with 95 °C for 30 s, 62 °C for 30 s, 72 °C for 30 s for RAGEΔ and 95 °C for 30 s, 62 °C for 30 s, 72 °C for 40 s for sRAGEΔ in additional 30 cycles. Human 18S rRNA gene was used as the internal control to calibrate the measurement. For western blot, the antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and the chemicals used in this study were purchased from Sigma–Aldrich (St. Louis, MO). In western blot, total proteins were isolated from IP region of two AD and two control subjects, respectively. Fifty microgram total proteins were loaded. Other related procedures are followed standard protocols or manufacturer's manuals. The Student's *t*-test was used in statistical analysis and significance was set at $^{*}p < 0.05$ and $^{**}p < 0.01$.

3. Results

Full length RAGE protein has 404 amino acids, and RAGEΔ is an isoform with 16 amino acids deleted in the intracellular domain that misses four amino acids (GEER) from exon 10 and 12 amino acids (KAPENQEEEEER) from exon 11 [15]. RAGEΔ has not been detected in other studies [8,14]. The deletion contains three positive charged amino acids and eight negative charged amino acids, which largely reduced the stability, hydrophobicity and interacting capacity of the intracellular domain (Fig. 1). For example, after the deletion the estimated half-life of the intracellular domain reduces from 30 h to 4.4 h, and the instability index (II) reduces from 120.41 to 99.51. Meanwhile, the aliphatic index changes from 23.79 to 45.38, the grand average of hydropathicity changes from −2.345 to −1.531 and the theoretical pI changes from 3.98 to 3.51 (www.expasy.org/tools).

The mRNA were isolated from different brain regions of the human specimens and used for gene expression measurement. The most significant difference was found in IP ($p = 0.007$) and hippocampus ($p = 0.034$) regions between control and AD subjects. Meanwhile no significant difference was detected in SMTG and cerebellum regions between control and AD subjects. In both hippocampus and IP regions the expression level of RAGEΔ was decreased significantly in AD subjects compared with control subjects (Fig. 2). On the other hand, the expression level of RAGEΔ in cerebellum was generally higher than that in other detected brain regions, with significantly higher RAGEΔ level in cerebellum than hippocampus and SMTG regions in control, and significantly higher RAGEΔ level in cerebellum than hippocampus, IP and SMTG regions in AD subjects (Fig. 2).

The isoform sRAGEΔ has no transmembrane domain and intracellular domain just like secretory RAGE (sRAGE, or soluble RAGE, RAGE-v1 [8]), which is no longer attached on cytoplasm membrane (Fig. 1). In addition it misses five amino acids (VPNEK) from exon 5 and seven amino acids (GVSVKEQ) from exon 6 [15]. This 12 amino acids deletion is located in the first constant Ig-homolog domain (C2), which connects the variable Ig domain and the second constant Ig domain (Fig. 1). There are both positive and negative charged residues (K and E, two each) in this deleted fragment, resulting in ∼1.3 KD molecular weight loss and hydrophobicity decrease, might interrupt ligands binding capacity in extracellular space (http://bioinf.cs.ucl.ac.uk/psipred/). Similar to RAGEΔ, sRAGEΔ has not yet been reported in other studies [8,14] and is unique in human brain tissue [15].

The expression level of sRAGEΔ was significantly decreased in all the detected brain regions in AD subjects compared with control subjects (Fig. 3). The expression level of sRAGEΔ in cerebellum showed significant lower level in AD subjects than control subjects. Such difference had not been detected in RAGEΔ and other AS isoforms in cerebellum (Fig. 2 and unpublished data). The expression level of sRAGEΔ in both AD and control samples is significantly