Review Article

Pathological Role of D-amino Acid-Containing Proteins and Advanced Glycation End Products in the Development of Age-Related Macular Degeneration

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Abstract

Age-related macular degeneration has become a leading cause of blindness in most developed countries worldwide. In the early phase of the disease, abnormal, yellow-colored material called “drusen” appears between the retinal pigment epithelial cells and Bruch’s membrane. Immunohistochemical studies have confirmed that drusen contain D-amino acid-containing proteins and advanced glycation end products. In addition, retinal pigment epithelial cells express a receptor for AGEs (RAGE). These findings indicate that persistent interaction between AGEs and RAGE is involved in the development of age-related macular degeneration.

Keywords: D-amino acids, AGEs, RAGE, age-related macular degeneration

Introduction

Global estimates place the number of people suffering from blindness worldwide at 50 million or more. While causes of blindness vary substantially by country, the number one cause worldwide is cataracts, accounting for 50% of all cases of blindness. However, because cataracts are treatable with surgery, the ratio of cataracts as a cause of blindness is relatively low in many of the wealthier, more developed countries. Instead, age-related macular degeneration (AMD) is the number one cause of loss of sight in developed countries, with ratios of 70% among Caucasians in the United States, 58% in Netherlands, and 50% in Australia. However, despite its importance as an ocular disease, little is known regarding the pathogenesis of AMD.

Reducing the number of patients suffering from blindness due to AMD will require further clarification of the pathogenesis of this disease and subsequent development of prevention and treatment methods. In the present study, we describe the post-translation modified proteins and D-amino acids, and the condition of AMD, focusing on advanced glycation end products (AGEs).

AMD as a representative age-related alteration

Many serious ocular diseases that lead to loss of sight, including cataracts and glaucoma, are closely linked with age (Fig. 1).

Fig. 1. The cause of sight loss in the world
The main cause to sight loss in the world is closely associated with age-related diseases such as cataract, glaucoma and Age-related macular degeneration (AMD).

Other factors suspected to be involved in the pathogenesis of AMD are history of smoking, arteriosclerosis, ultraviolet ray exposure, and genetics, among others. However, as mentioned above, the aging process is deeply involved in the pathogenesis of AMD1-3).
Abnormal accumulated proteins in AMD

Symptoms of AMD vary in severity from negligible change without visual impairment to the final stage of blindness. In the early stages, abnormal accumulation of yellow-brownish-colored proteins, known as drusen, without angiogenesis can be noted in the macular area. Histological examination has shown that drusen are deposited between the retinal pigment epithelium and basal membrane (Fig. 2).

On disease progression, however, angiogenesis occurs in the macular area, causing exudation and hemorrhage and leading to impairment of visual acuity. This finding strongly suggests the association between drusen and AMD, underscoring the need to examine the composition of drusen to clarify the pathogenesis of AMD.

One method of analyzing the composition of drusen involves comprehensive identification of all proteins contained in the accumulated material. Crabb et al. conducted proteome analysis of these proteins and identified 129 kinds of proteins, including clusterin, TIMP3, and albumin. In their proteome analysis of drusen using a Macaca fascicularis model of AMD, Umeda et al. identified proteins such as annexin V, clusterin, complement components, and vitronectin. Altogether, a large number of proteins contained in drusen have been identified by comprehensive analysis of the composition. However, precisely which protein or proteins play an important role in the pathogenesis of AMD remains uncertain.

Another method of analyzing the composition of drusen involves examining post-translational modifications of proteins. After translation, proteins are modified in two different manners: an essential reaction mediated by specific enzymes (such as glycosylation and phosphorylation) which enable proteins to function physiologically, or a reaction not mediated by any enzyme, such as glycation or racemization. Prevalence of proteins receiving non-enzymatic post-translational modifications often increases with aging and is thought to be closely related to age-associated changes. Here, we focused on AGES and D-amino acids resulting from racemization with non-enzymatic post-translational modification.

D-amino acids in AMD

Chemical synthesis of amino acids produces a 1:1 ratio of D- and L-amino acids. However, D-amino acids are believed to have been excluded in the process of evolution, as all proteins in living things on earth are composed of L-amino acids. In addition, D-amino acids were previously recognized only in fossils and were not believed to exist in the human body, as the half-life of the reaction converting L- into D-amino acids is several thousand years (Fig. 3). However, recent studies have determined the involvement of D-amino acid-containing proteins in diseases closely related to aging process and age-related disorders, such as cataracts and Alzheimer’s disease. Proteins containing D-amino acids have therefore come to be suspected of playing an important role in age-related alterations.

Given the present lack of a method for comprehensively analyzing D-amino acid-containing proteins, we synthesized a peptide that contained the array before and behind 151Asp of the crystalline molecule frequently converted from L- to D-Asp in the crystalline lens. The peptide consisted of Gly-Leu-D-β-Asp-Ala-Thr-Gly-Leu-D-β-Asp-Ala-Thr-Gly-Leu-D-β-Asp-Ala-Thr, and we constructed a polyclonal antibody against this sequence. We then established a method to comprehensively examine D-Asp-containing proteins using this antibody. In this manner, we clarified that drusen and hypertrophic Bruch’s membrane in the eyeballs of elderly individuals were comprised of proteins containing D-Asp (Fig. 4).

At present, little is known regarding the precise biological role of D-amino acid-containing proteins. However, these proteins’ structures differ greatly from those of L-amino acid-containing proteins due to the change in bond angle with the adjacent amino acid which occurs on chirality conversion. These conformational changes of protein molecules may result in reduced protein function, and indeed, the chaperone activity of the crystalline molecule decreases with increasing levels of D-amino acids in the molecule, which is the main protein composing the crystalline lens, with aging. Similarly, this finding suggests that decline of the protein function may be involved in the pathogenesis of AMD.

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**Fig. 2.** Eye fundus image and histology of AMD

In the early stage of AMD, drusen is noted in the macular region as yellow-brownish accumulation products. Histologically, drusen is recognized in the area between the retinal pigment epithelium and Bruch membrane (arrows).
**AGEs in AMD**

When a protein contains a reducing sugar such as glucose or fructose, an Amadori product is slowly generated, and through the complicated reactions of oxidation, dehydration, and condensation, among others, AGEs are subsequently generated. Because this process is irreversible, AGE formation in the protein progresses slowly and steadily over a long period of time, with AGEs adopting a peculiar structure independent of the type of sugar or proteins used as materials.

The structures of several AGEs have been determined, including CML (Nε-(carboxy)methyl-L-lysine), CEL (Nε-(carboxy)ethyl-L-lysine), and pentosidine. Common structures of AGEs have been identified both *in vitro* and *in vivo* and are known to be present in various organs associated with age-related diseases or diabetes mellitus. Examination of AGE localization in AMD as the representative disease for age-related alterations has clearly shown that drusen is comprised of accumulated proteins which abundantly contain AGEs (Fig. 5). AGEs have also been observed in the hypertrophic area of Bruch’s membrane in elderly subjects. Drusen may thus be deposited not only on aging but also by hemorrhage and exudation, implying its involvement in the pathogenesis of AMD. Autoantibodies against AGEs are occasionally produced in the body, and accumulation of drusen in the macular region may thereby cause a local inflammatory reaction. In addition, AGEs are known to exert biological action by interacting with AGE receptors. The pathogenesis of AMD is outlined with respect to interaction between AGEs and AGE receptors in the following section.
**Pathogenesis of AMD at the molecular level**

AGEs and RAGE are mainly described in the context of their relation to the pathogenesis of AMD. Inflammatory reactions and angiogenesis are known to play a key role in AMD, and these changes may be due to continuous contact between the retinal pigment epithelium expressing RAGE and drusen abundantly containing AGEs (Fig. 6).

This finding suggests that the possibility for prevention or treatment of AMD by inhibiting the interaction between AGEs and RAGE. The antioxidants aminoguanidine and pyridoxamine have been experimentally proven to inhibit diabetic complications in organs by inhibiting generation of AGEs. In addition, administration of soluble RAGE, an extracellular domain of RAGE, has been proven to prevent diabetic complications by inhibiting the interaction between AGEs and AGE receptors.

However, whether or not newly developed medicines targeting AGEs and RAGE can prevent or treat AMD based on these points remains to be determined.

Here, we showed that both AGEs and D-amino acids play key roles in age-related alterations. While receptors to D-amino acids and AGEs in Age-Related Macular Degeneration
involved in the pathogenesis of AMD is therefore necessary. Further clarification of how D-amino acid-containing proteins are both known to be deeply involved in age-related alterations. 

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