Available online at www.sciencedirect.com
ScienceDirect
Experimental Eye Research 86 (2008) 856–859
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Letter to the Editor

Immunohistochemical analysis of aldehyde-modified proteins in drusen in cynomolgus monkeys (*Macaca fascicularis*)☆

Sachiko Kaidzu [a], Masaki Tanito [a,*], Akihiro Ohira [a], Shinsuke Umeda [b], Michihiro Suzuki [c], Yasuhiro Yoshikawa [d], Takeshi Iwata [b]

[a] *Department of Ophthalmology, Shimane University Faculty of Medicine, 89-1 Enya, Izumo, Shimane 693-8501, Japan*
[b] *National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center, Tokyo, Japan*
[c] *Corporation for Production and Research of Laboratory Primates, Ibaraki, Japan*
[d] *Department of Biomedical Science, Graduate School of Agricultural and Life Science, University of Tokyo, Tokyo, Japan*

Received 20 December 2007; accepted in revised form 14 January 2008
Available online 31 January 2008

Abstract

Protein modifications resulting from reactive aldehydes are thought to be involved in the pathogenesis of various degenerative diseases. Aged cynomolgus monkey (*Macaca fascicularis*) spontaneously develop drusen in the macula, consistent with the phenotype observed in early-stage age-related macular degeneration (AMD), indicating that this animal is an optimum model for AMD. In retinal sections from three monkeys with macular degeneration, regardless of their size, drusen were consistently positive with immunohistochemical labeling against protein modifications by 4-hydroxynonenal and 4-hydroxyhexenal, end products of non-enzymatic oxidation of n-6 and n-3 polyunsaturated fatty acids, respectively. Positive labeling for both modifications was observed in the ganglion cell layer, the inner nuclear layer, the outer nuclear layer, and the retinal pigment epithelium. However, no consistent differences in location or intensity of the labeling were observed between monkeys with normal macula and macular degeneration. The results suggest a possible association between drusen formation and protein modifications by aldehydes in the pathogenesis of AMD.

Keywords: aldehydes; 4-hydroxynonenal; 4-hydroxyhexenal; cynomolgus monkey; drusen; age-related macular degeneration; protein modification

Age-related macular degeneration (AMD) is the leading cause of legal blindness in elderly individuals in industrialized countries (Fine et al., 2000). Accumulation of extracellular deposits between the retinal pigment epithelium (RPE) and Bruch's membrane, referred to as drusen, is regarded as a hallmark risk factor for development of AMD (de Jong, 2006). Various lipids, polysaccharides, and glycosaminoglycans have been identified as constituents of drusen (Hageman et al., 2001). Recent studies have revealed that drusen contains various protein molecules that are related to inflammation, immune responses, and oxidative stresses (Mullins et al., 2000; Crabb et al., 2002); yet the mechanism of formation is not fully understood.

Aged monkeys spontaneously develop macular degenerative changes such as pigment mottling, hyperpigmentation or hypopigmentation, and drusen in the macula, consistent with the phenotype observed in early-stage AMD (Stafford et al., 1984; Ishibashi et al., 1986). Previous proteomic analysis indicated that a number of protein components are common in drusen from monkeys and humans (Crabb et al., 2002; Umeda et al., 2005). Thus, these animals are thought to be an optimum animal model for AMD.

4-Hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE) are α,β-unsaturated aldehydes that are end products of non-enzymatic oxidation of n-6 and n-3 polyunsaturated fatty acids, respectively (Esterbauer, 1993). These highly reactive aldehydes can react readily with histidine, cysteine, or lysine

☆ The authors have no proprietary interest in any aspect of this report.

* Corresponding author. Tel.: +81 853 20 2284; fax: +81 853 20 2278.
E-mail addresses: tanito-oph@umin.ac.jp, mtanito@med.shimane-u.ac.jp (M. Tanito).

0014-4835/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2008.01.016

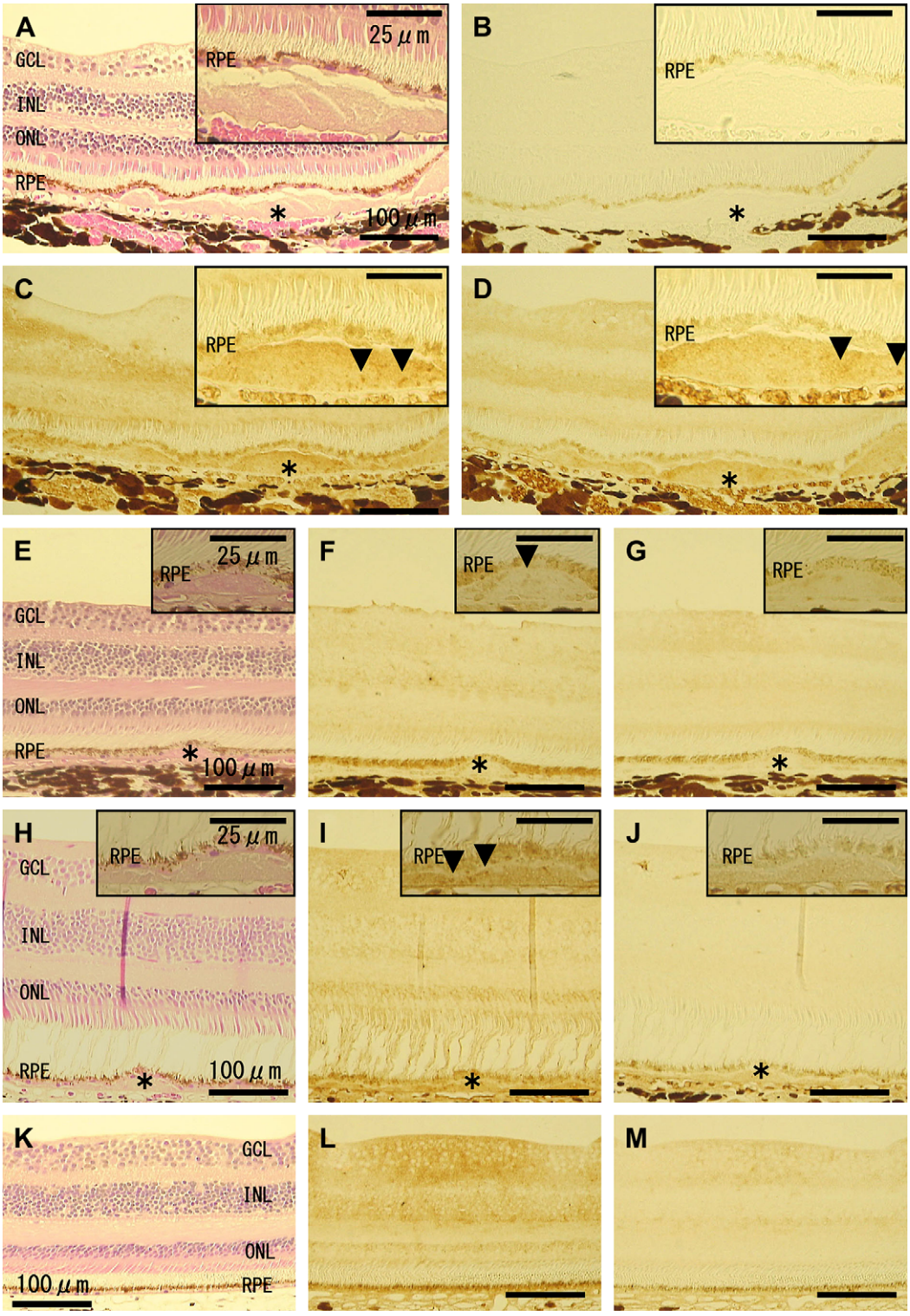

Fig. 1. Retinal sections from monkeys with macular degeneration and normal macula. Representative retinal sections from three monkeys with macular degeneration (panels A–D, E–G, and H–J, respectively) and those from a monkey with a normal macula (panels K–M) are shown. Representative sections stained with hematoxylin and eosin (panels A, E, H, and K) labeled with normal mouse IgG (panel B), 4-HNE-modified proteins (panels C, F, I, and L) and 4-HHE-modified proteins (panels D, G, J, and M). Asterisks and arrowheads indicate drusen and granular labeling, respectively. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; and RPE, retinal pigment epithelium.