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Age-dependent changes in the structure, composition and biophysical properties of a human basement membrane

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ABSTRACT

Basement membranes (BMs) are considered to be uniform, approximately 100 nm-thin extracellular matrix sheets that serve as a substrate for epithelial cells, endothelial cells and myotubes. To find out whether BMs maintain their ultrastructure, protein composition and biophysical properties throughout life the natural aging history of the human inner limiting membranes (ILM) was investigated. The ILM is a BM at the vitreal surface of the retina that connects the retina with the vitreous. Transmission electron microscopy (TEM) showed that the ILM steadily increases in thickness from 70 nm at fetal stages to several microns at age 90. By the age of 20, the ILM loses its laminated structure to become an amorphous and very irregular extracellular matrix layer. Atomic force microscopy (AFM) showed that the native, hydrated ILMs are on average 4-fold thicker than the dehydrated ILMs as seen by TEM and that their thickness is prominently determined by its water-binding proteoglycans. The morphological changes are accompanied by age-related changes in the biochemical composition, whereby the relative concentrations of collagen IV and agrin increase, and the concentration of laminin decreases with age. Force-indentation measurements by AFM also showed that ILMs become increasingly stiffer with advancing age. The data suggest that BMs from other human tissues may undergo similar age-related changes.

1. Introduction

Basement membranes (BMs) are extracellular matrix sheets that are found at the base of every epithelium, at the surface of all muscle fibers and at the basal surface of vascular endothelial cells. BMs are composed of extracellular matrix proteins that include members of the laminin, nidogen collagen IV and proteoglycan families (Timpl and Brown, 1996; Erickson and Couchman, 2000). The commonly accepted model proposes that two networks of polymerized laminins and crosslinked collagen IVs constitute the basic frame-work of every BM. Deletion or mutations of BM proteins result in embryonic lethality (Smyth et al., 1999; Willem et al., 2001; Costell et al., 1999; Arikawa-Hirasawa et al., 1999; Poeschl et al., 2004; Bader et al., 2005) or, postnatally, in muscular dystrophy, paralysis (Gautam et al., 1996), vascular disruption (Gould et al., 2005; 2007), and eye and brain abnormalities (Sertie et al. 2000; Fukai et al., 2002; Halfter et al., 2002; 2005a; Lee and Gross, 2007). The fact that many of the BM protein mutations cause vascular disruptions with increasing blood pressure during later embryogenesis showed that BMs have an important role in the mechanical stability of tissue walls (Candiello et al., 2007).

Most studies on BMs were conducted in mice, rats, fish and *Drosophila*, laboratory animals that have a very limited life span. They show that BMs are rather uniform and thin ECM sheets of less than 100 nm thickness. In the longer-lived humans, however, many extracellular matrix tissues undergo age-related structural and compositional changes that only become obvious after decades of life. It is well conceivable that human BMs also undergo age-related structural and compositional changes and that these changes are only minor or even undetectable in most laboratory animals. To this end, we investigated the age-related structural, compositional and biomechanical changes of a human BM, the inner limiting membrane (ILM). The inner limiting membrane is a BM that is located at the border between the retinal neuroepithelium and the vitreous body (VB). Proteome analysis of the chick ILM showed that it is composed of the BM-typical ECM proteins that are found in other BMs as well (Candiello et al., 2007; Balasubramani et al., in press). Analysis of mice, zebra fish and humans with mutations or deletions of BM proteins (Zenker et al., 2004; Halfter et al., 2005a; Semina et al., 2006; Lee and Gross 2007) or their receptors (Georges-Labouesse, 1998; Satz et al., 2008) has shown that the ILM is essential in retinal histogenesis by establishing a substrate for the neuroepithelial endfoot processes to attach to. In cases where the ILM was defective, the Muller cell processes were retracted, and the histogenesis of the retinal cell layers was greatly disrupted, eventually resulting in retinal dysplasia, retinal ectopia and a massive loss of

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ganglion cells and axons (Halfter et al., 2005a, Semina et al., 2006; Lee and Gross 2007). The ILMs can be isolated, and it is one of the few BMs that are accessible for direct biochemical analysis. In addition, the ILM can be flat-mounted on glass slides and is thereby suitable for biomechanical measurements using atomic force microscopy (AFM; Candiello et al., 2007). Thus, the ILM provides unique experimental advantages over most other BMs that are inseparable from adjacent connective tissues and that are difficult to mount for biophysical examination.

The present study shows that the human ILM undergoes age-dependent alterations that include a dramatic increase in thickness, a loss of the typical BM ultrastructure, an increase in stiffness and age-related changes in its biochemical composition. We propose that BMs from other tissues undergo similar age-related changes that may contribute to an increase in disease frequency at older age.

2. Results

2.1. Location, protein composition and morphology of the fetal human ILM

The ILM is located at the vitreal border of the retina (Fig. 1A). It is one of 6 BMs of the eye, which include the lens capsule, the BMs of the cornea, the BM of the pigment epithelium and the BMs of the intravitral hyaloidal vessels. The protein composition of the ILM from human fetal eyes was analyzed by immunocytochemistry using antibodies against basement membrane-specific proteins. Consistent with earlier reports, labeling of the ILM and the other ocular BMs was recorded after staining with antibodies to collagen IV (Fig. 1A), laminin-1 (Fig. 1B, C), nidogen-1, perlecan, collagen XVIII and agrin (Halfter et al., 2005b, 2008). The immunocytochemistry was reproduced with 6 fetal human eyes and with 10 adult human eyes from ages 22 to 86. Transmission electron microscopy (TEM) of 10 and 20-week fetal human retina samples showed that the ILM of the fetal human eye has the typical ultrastructure of a standard BM with a central lamina densa and an outer and inner lamina rara (Fig. 2A). The ILM of the 10 and 20-week fetal human eye has a thickness of 70 ± 7 nm ($n = 6$ pairs of fetal eyes).

2.2. Structure and composition of the adult human ILM

To find out whether the ILM undergoes age-related structural changes thin sections of the retina from fetal and adult human eyes were examined by TEM. Since the structure of the adult human ILM varies depending on the location of the retina (Foos, 1972), all samples were taken from the dorso-central retina (Fig. 2E). Different to the 70 nm-thin and very even ILM at 20 weeks of gestation (Fig. 2A), the adult human ILM loses its distinctly layered ultrastructure and becomes gradually thicker and more irregular with advancing age. By age 22, the thickness of the ILM had increased to 300–350 nm and had lost its lamina densa and rara substructures (Fig. 2B). By age 50, the ILM had further increased in thickness and the retinal surface of the ILM had long extensions deep into the retina (Fig. 2C). These indentations were even more pronounced at older age. The vitreal surface of the ILM remained smooth at all ages. By age 83, the ILM thickness had increased to over 1500 nm (Fig. 2D), over 20 fold relative to the fetal ILM. The graph in Fig. 2F shows the age-dependent increase in human ILM thickness. The increasingly larger standard deviations at older age accounted for the more frequent and more extensive indentation of the ILM at its retinal surface.

2.3. Isolation of the adult human ILM and the protein composition of the aging human ILM

To analyze the protein composition and to probe the biomechanical qualities of the adult human ILM we devised a method to isolate the ILM from adult human retina. The procedure takes advantage of the fact that BMs are insoluble in detergent (Meezan et al., 1975; Duhamel et al., 1983; Halfter and von Boxberg, 1992): following the dissolution of the retinal cells in Triton-X-100 and deoxycholate the ILMs were collected from the detergent solution under a dissecting microscope using dark-field illumination (Fig. 3A, insert). TEM micrographs (Fig. 3A) showed that the isolated ILMs were sheets of extracellular matrix, free of cellular debris and organelles. Further, the vitreal surface of the sheets was smooth and even, whereas the retinal surface had a very irregular appearance, identical to the retinal surface of the ILM in situ (Fig. 3B).

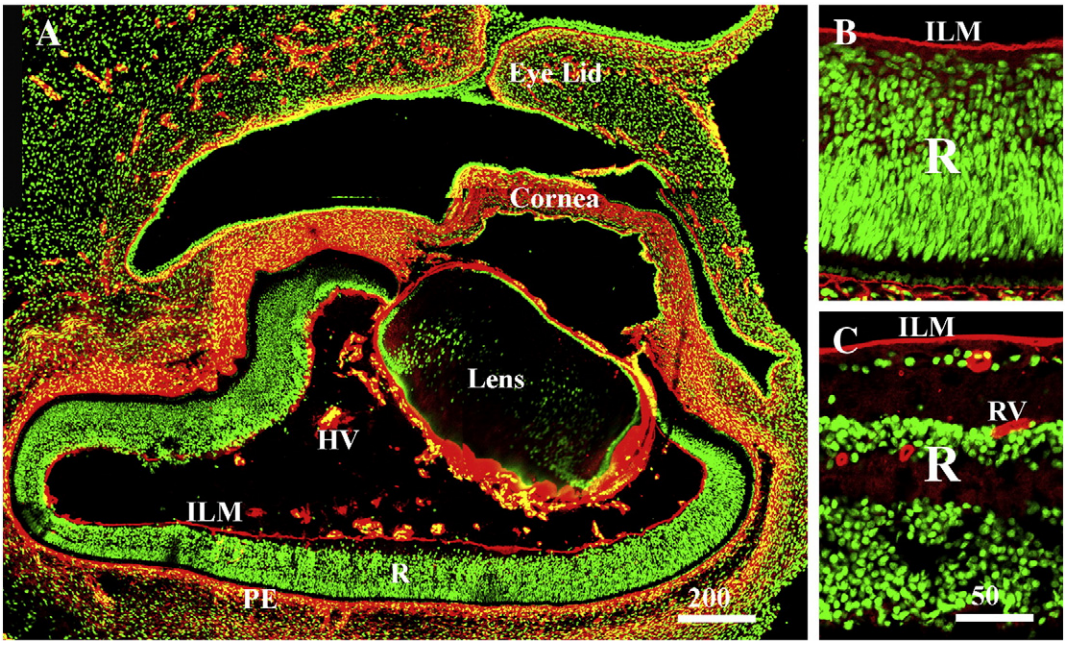

Fig. 1. BMs in the fetal human eye. The low power overview of an 10-week fetal human stained for collagen IV (red) shows the BM of the lens, the hyaloids vessels in the vitreous (HV), the pigment epithelium (PE), the ILM, and the vascular BMs in the eye lids. The corneal stroma is also labeled. High power micrographs of the 10 week fetal retina (B) show strong labeling of the ILM and the BM of the pigment epithelium and the choroid for laminin-111. Staining of the adult human retina for laminin-1 (1C) shows the ILM and the BMs of the retinal blood vessels (RV). Bars: a: 200 µm; B, C: 50 µm.