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Review

Imaging of cellular aging in human retinal blood vessels

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ABSTRACT

To date two main aging vascular lesions have been reported in elderly human retinas: acellular capillaries and microaneurysms. However, their exact mechanism of formation remains unclear. Using high resolution microscopy techniques we revise cellular alterations observed in aged human retinal vessels, such as lipofuscin accumulation, caveolae malfunction, blood basement membrane disruption and enhanced apoptosis that could trigger the development of these aging vascular lesions. Moreover, we have generated a set of original images comparing retinal vasculature between middle and old aged healthy humans to show in a comprehensive manner the main structural and ultrastructural alterations occurred during age in retinal blood vessels.

1. Introduction

Population aging is a global phenomenon of the 21st century. The number of seniors (65 and older) is projected to triplicate by 2050 (United Nations, Department of Economic and Social affairs, 2013). The biological process of aging is a result of genetic and environmental interactions that cause molecular damage and impaired function of cells (Zhang, 2007; López-Otín et al., 2013). The aged organism is no longer capable of maintaining the homeostatic balance between cell loss and cellular renewal, which causes accumulation of damaged cells, metabolic toxins, and waste products (Ardeljan and Chan, 2013).

These age-related changes in cellular homeostasis are also reflected in the retinal circulatory system. In the retina there is a compromise between optimal visual function and optimal oxygenation (Funk, 1997). Retinal capillaries have a relative sparse distribution and their size is small in order to minimize optical interference with the light path (Ruberte, 2010). Hence, the blood flow volume in the retina is relatively low (Hickam and Frayser, 1965; Alm and Bill, 1973). This fact, together with the high oxygen consumption of the retinal tissue (Alm and Bill, 1973), would facilitate the development of retinal hypoxia when the vascular bed is altered, such as in aging. Little data is available on the morphological alterations that occur during normal aging of retinal microvasculature. However, altered ocular blood flow, loss of retinal blood barrier integrity and reduced vascular branching are some of the alterations observed in healthy individuals as they age (Van-Kirk et al., 2011; MacIntyre et al., 2012; Leung et al., 2003). Moreover, these aging modifications also render aged blood vessels more susceptible to the damaging effects of age-related diseases, such as diabetic retinopathy, hypertensive retinopathy, and age-related macular degeneration (Nag and Wadhwa, 2012; Ungvari et al., 2010).

In this review, we compare retinal vasculature from middle and old aged healthy humans. The goal is to present in a comprehensive manner the main alterations occurred with age in retinal blood

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vessels using high resolution microscopy techniques. We provide morphological basis to better understand the aging process, and highlight the differences and similarities between normal aging and age-related ocular diseases.

2. Retinal vasculature in elderly humans

The human retina has two main sources of blood supply: the central retinal artery and the choroidal blood vessels (Anderson & McIntosh et al., 1967; Kur et al., 2012). The central retinal artery enters the eye through the optic disc and divides into four main retinal arterioles that branch out towards the retinal periphery into smaller arteriolar branches, the pre-capillary arterioles, which feed the capillary network (Fig. 1A) (Pournaras et al., 2008; Anderson and Mcintosh, 1967). The retinal venous system has a similar arrangement and the main retinal venules drain into the central retinal venule that leaves the eye through the optic disc (Kur et al., 2012). Furthermore, human retinas have an avascular region, the fovea, where visual acuity is maximal (Fig. 1A) (Kur et al., 2012; Wise, 1971).

Throughout the cellular retinal layers, blood vessels are organized in three interconnected vascular plexi (Fig. 1B). The superficial plexus, located in the ganglion cell layer, consists of large retinal arterioles and venules. The deep plexus is formed only by capillaries and is situated between the inner and outer nuclear layers (Kur et al., 2012). The intermediate plexus is less prominent and consists of short capillaries that connect the superficial and deep plexi (Kur et al., 2012; Hughes et al., 2000).

The walls of retinal blood vessels are composed by three distinct tunicae: intima, media and adventitia (Fig. 1C) (Ramos et al., 2013). The tunica intima is the innermost and is formed by a single layer of endothelial cells in direct contact with the vascular lumen (Wise, 1971). The tunica media displays multiple layers of smooth muscle cells and pericytes. Finally, surrounding the surface of endothelial cells, pericytes and smooth muscle cells there is an extracellular matrix sheet known as the blood basement membrane, which forms externally the tunica adventitia (Candiello et al., 2010). A recent study performed by Muraoka et al. (2013)

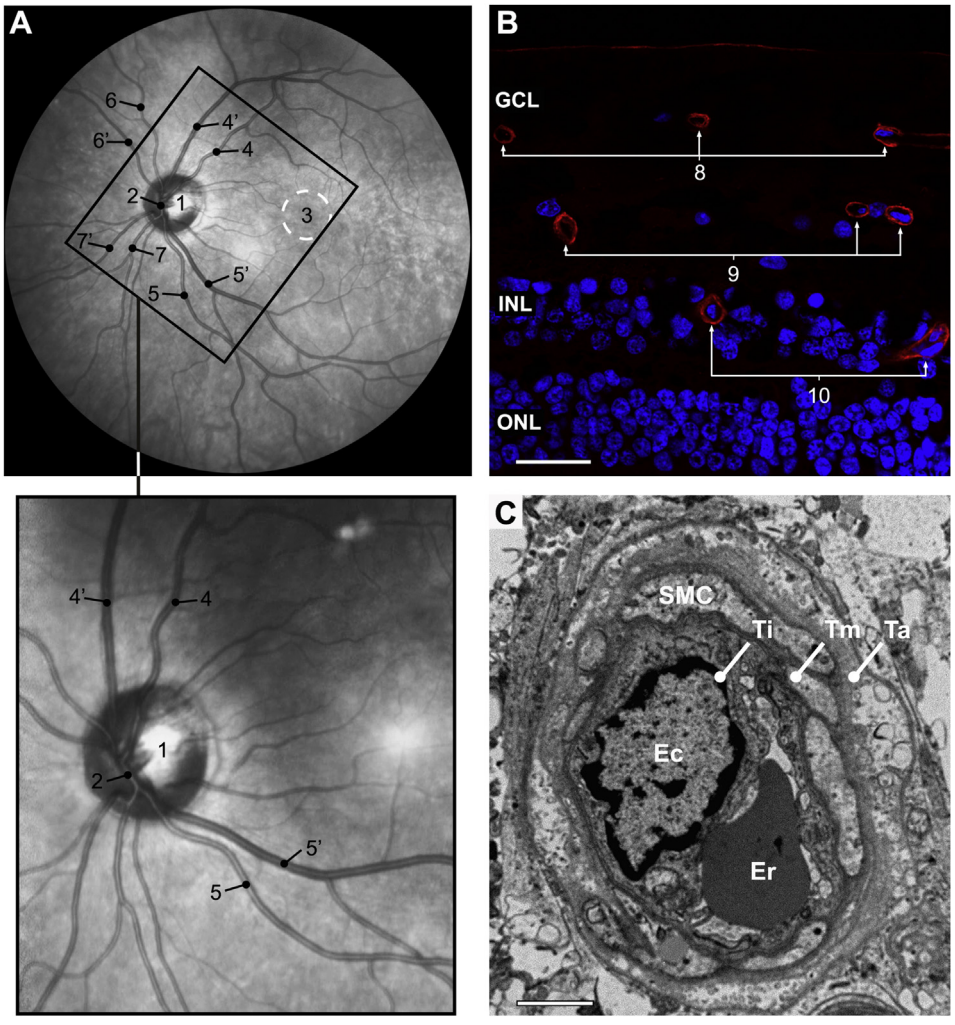

Fig. 1. Vascularization of the human retina. (A) Eye fundus image showing an angiogram acquired with a Scan Laser Ophthalmoscope. Higher magnification illustrates how the central retinal artery emerges from the optic disc and branches into four main arterioles, which in turn divide into smaller arterioles directed towards the retinal periphery. The retinal venules run parallel to the arterioles towards the optic disc. (B) Topography of retinal vascular plexi. Paraffin section of a human retina immunohistochemically labeled with anti-collagen IV (red). Nuclei counterstained with Hoechst (blue). (C) TEM image from a retinal arteriole showing three tunicae surrounding the vascular lumen. 1: Optic disc; 2: Central retinal artery; 3: Fovea; 4: Superotemporal arteriole; 4′: Superotemporal venule; 5: Inferotemporal arteriole; 5′: Inferotemporal venule; 6: Superonasal arteriole; 6′: Superonasal venule; 7: Inferonasal arteriole; 7′: Inferonasal venule; 8: Superficial retinal plexus; 9: Intermediate retinal plexus; 10: Deep retinal plexus. GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer. Ec: Endothelial cell; Er: Erythrocyte; SMC: Smooth muscle cell; Ta: Tunica adventitia; Tm: Tunica media; Ti: Tunica intima. Scale bars: 30 μm (B) and 1.5 μm (C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)