Collagens and retinal Müller cells in healthy and diseased vitreoretinal interface

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Summary

Chapter 1. General introduction The vitreoretinal interface encompasses the cortical vitreous, inner limiting membrane (ILM) and the endfeet of retinal Müller cells. It is a highly organized and complex extracellular matrix structure composed of collagens, proteoglycans (PGs) and glycoproteins (GPs). With age, the vitreoretinal interface undergoes a dynamic remodelling process which is related to certain ageing phenomena (e.g. synchysis, syneresis, and posterior vitreous detachment). Furthermore, the remodelling of the ECM at the vitreoretinal interface during ageing and fibrotic processes may regulate the pathogenesis of certain vitreoretinal diseases.

Chapter 2. A review of the literature on idiopathic epiretinal membrane (iERM) is given. Epidemiologic studies report on a relationship between iERM prevalence and increasing age, posterior vitreous detachment (PVD), ethnic groups etc. Clinically, iERM progresses through different stages characterized by an increased thickness and wrinkling of the membrane. Diagnostic optical coherent tomography (OCT) procedures have been refined over the last decades and OCT aspects may be informative in predicting visual outcomes after surgical removal of the iERM. Pathophysiologically, iERM formation is a fibrotic process in which myofibroblast formation and the deposition of newly formed collagens (type I, III, IV and VI collagens) play key roles. Myofibroblasts may have multiple origins, and their formation is stimulated by cytokines in the micro environment, as well as by cues obtained from structural macromolecules in the ECM. Anomalous PVD may be a key event initiating the formation of iERM. The age-related accumulation of advanced glycation end products may contribute to anomalous PVD formation and may also influence the mechanical properties of the iERM. The understanding of molecular mechanisms underlying the pro-fibrotic effects of ECM components and

the mechanical cues permit the construction of a plausible sequence of events that lead to the development of iERM.

Chapter 3. The ultrastructural localization of type II, IV and VI collagens in the adult human vitreoretinal interface of five human donor eyes was evaluated by transmission electron microscopy using immunogold labeling. In the pre-equatorial region, we observed densely packed vitreous lamellae with a partly intraretinal course containing type II and VI collagens, reticular structures containing type IV and VI collagens and a thin inner limiting membrane (ILM) containing type IV and VI collagens in a linear distribution pattern. The presence of type VI collagen in vitreous lamellae penetrating the ILM into the superficial retina suggests that type VI collagen is a critical component involved in the organization of vitreous lamellae and the adhesion of the vitreous fibers to the surjacent retinal matrix and retinal cells. From the anterior to the posterior retina, the linear labeling pattern of type IV and VI collagens gradually became more diffuse, and labeling could be observed throughout the entire thickness of the ILM. The close co-distribution of type IV and VI collagens in the ILM suggests that both types of collagen important structural components of the ILM. The reticular labeling patterns observed in the anterior vitreous are highly similar to labeling patterns of blood vessel walls. In the anterior vitreous, they may represent remnants of the regressed embryonic hyaloid blood vessel system.

Chapter 4. Epiretinal membranes (ERMs) from idiopathic macular hole patients were processed for flat-mount and immuno-histochemistry. ERM is a glail fibrillary acidic protein (GFAP)-positive gliotic and fibrotic scar which contains newly formed type I, III and V collagens. Type VI collagen was not observed. Co-localization studies found cells co-expressing GFAP/ cellular retinaldehyde-binding protein (CRALBP), GFAP/ α-smooth muscle actin (α-SMA), and α-SMA/CRALBP, which is consistent with transdifferentiation of Müller cells into a fibroblast- and myofibroblast-like

phenotype. The clinically significant ERMs can be divided into two groups according to the amount of cells in the ERM: sparse cellular proliferation and dense cellular proliferation. The latter group is associated with a higher chance of surgical difficulty during ILM peeling. The retinal Müller cell is one of the important cell types that are involved in ERM formation in idiopathic macular hole.

Chapter 5. Epiretinal membranes from idiopathic epiretinal membrane (iERM) patients were assessed by flat-mount immunohistochemistry. The presence of type VI collagen was found in these ERMs. The co-localization of GFAP/CRALBP and GFAP/α-SMA in the epiretinal cells indicated a dynamic process of Müller cell activation and transformation. Furthermore, in vitro studies showed that TGF-β1 induces an up-regulation of α-SMA in retinal Müller cells while the expression of type I, II and VI collagens in the cells containing α-SMA positive stress fibers was significantly down-regulated.

Chapter 6. Retinal Müller cells were cultured on tunable polyacrylamide gels with various elastic moduli. The results showed that an increase in substrate stiffness promotes the myofibroblast transdifferentiation of retinal Müller cells in response to stimulation with transforming growth factor-beta (TGF-β). This evidence suggests that retinal Müller cells that come into contact with a stiffer extracellular matrix such as that resulting from ageing or fibrotic processes, may be more susceptible to TGF-β stimulation. The latter may then result in an up-regulation of α-SMA and thus promote membrane contraction.