Chapter 7

General Discussion
Mechano-activation of Müller cells induced by vitreo-macular traction may be a common pathogenic factor in vitreo-macular diseases

The vitreoretinal interface is a dynamic extracellular matrix (ECM) structure which undergoes constant remodelling throughout a lifetime. Remodelling of the ECM at the vitreoretinal interface, both resulting from ageing and fibrotic changes, plays a significant role in the pathogenesis of many vitreoretinal disorders. Understanding the biochemical and biomechanical roles of aged ECM in fibrosis will permit the construction of a plausible sequence of events leading to the development of these sight-threatening conditions.

Based on current clinical and laboratory observations, we hypothesize that the age-related ECM remodelling of the vitreoretinal interface could result in an increased tissue stiffness and a prolonged vitreoretinal traction, which could trigger the proliferation, migration and transdifferentiation of Müller cells by mechanical traction. The mechanically activated Müller cells could further release and activate certain cytokines that eventually lead to the formation of an epiretinal membrane. Furthermore, increased tissue stiffness resulting from age-related ECM remodelling provides a profibrotic environment by activating profibrotic cytokines, such as transforming growth factor-β (TGF-β), which maintain the functional activity of myofibroblasts (Fig. 1).

Mechanosensitivity of Müller cells and its role in the pathogenesis of vitreoretinal diseases

With aging, a posterior vitreous detachment (PVD) develops progressively, which usually starts as a partial PVD at the perifoveal area. This may induce antero-posterior traction to the foveal area during eye movements, because of a strong persistent vitreo-macular adhesion.\textsuperscript{1, 2} The cortical vitreous remnants left on the macula after spontaneous PVD strongly suggest the presence of strong vitreo-
Figure 1 Schematic diagram of the proposed theory on the pathogenesis of epiretinal membrane associated with vitreo-macular traction. AGEs: advanced glycation end products; PVD: posterior vitreous detachment; TGF-β: transforming growth factor-β.

macular adhesion. It has been proposed that adhesion molecules, such as fibronectin, laminin, heparan sulphate proteoglycans and opticin, are responsible for the vitreoretinal adhesion by interacting with both type II collagens in the vitreous fibril and type IV collagens in the ILM.\textsuperscript{3-5} Additionally, the collagens identified by our group in the vitreoretinal interface, such as type VI, VII and XVIII collagens may be involved in the molecular mechanism of vitreoretinal adhesion.\textsuperscript{6} Furthermore, the age-related accumulation of advanced glycation end products (AGEs) and deposition of newly formed collagen may contribute to an increase in tractional forces by increasing the elastic modulus of the vitreoretinal ECM.
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Dynamic antero-posterior vitreo-macular traction has been well accepted as an important pathogenic factor in various macular diseases.\(^7\,^8\) The direct pulling force exerted by shrinking vitreous has been suggested to physically damage the retina and cause a defect in retinal tissue including the inner limiting membrane (ILM). Such an event can lead to the formation of an idiopathic macular hole or an idiopathic epiretinal membrane.\(^9\,^10\) Besides a direct mechanical impact, the traction force may also exert a biomechanical effect on the retina during the process of PVD. Müller cells are highly concentrated at the central foveal area and are suggested to be responsible for the foveal architecture and its structural integrity.\(^11\) Mechanical traction forces onto the fovea may activate these Müller cells, since they have been shown to be mechanically sensitive as aforementioned in Chapter 6.\(^12\,\text{ }^13\)

The mechanical sensitivity of Müller cells may play an important role at the initial stages of certain vitreo-macular diseases. As the principle retinal glial cells, Müller cells are responsible for the normal retinal functions and they are actively involved in retinal pathology.\(^14\) The gliotic response and proliferation of Müller cells can be triggered by retinal injury, vitreous hemorrhage, inflammatory factors, cytokines and growth factors released during blood-retinal barrier breakdown and mechanical traction from an anomalous posterior vitreous detachment.\(^15\) However, retinal tissue damage and blood-retinal barrier breakdown are not common in the early stages of certain vitreo-macular diseases, i.e. idiopathic macular hole, idiopathic epiretinal membrane and vitreo-macular traction syndrome. It is conceivable that the activation of Müller cells by mechanical traction may be the initial pathological change in these diseases. Furthermore, the subsequent gliotic scar formation may also partially be due to persistent vitreo-retinal adhesion and tangential traction because the increased tension could promote the formation of myofibroblasts.
General discussion

Mechanical stimuli can regulate the proliferation, migration and transdifferentiation of many types of cells. The signal transduction pathways which can be triggered by mechanical stimuli include mitogen-activated protein kinase pathway (MAP kinase), purinergic G protein-coupled receptors (P2Y receptors) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway. These signaling pathways have been shown to induce the expression of glial fibrillary acidic protein (GFAP), the hallmark of Müller cell activation. Lindqvist et al demonstrated that mechanical stretching can induce an immediate increase in intracellular Ca$^{2+}$ concentration and upregulation of basic fibroblast growth factor (bFGF), c-Fos and extracellular-signal-regulated kinases (ERK) in the Müller cells. Bringmann and Wiedemann suggested that the mechanical stress onto the Müller cells may trigger the activation of Ca$^{2+}$-dependent potassium channels which can stimulate Müller cell proliferation. Nonetheless, the molecules involved in the mechanical sensing process of Müller cells have not yet been clarified.

Integrins can mechanically stimulate MAP kinase and NF-κB pathways and are thus important candidates in mediating the mechanical cell to matrix signaling transduction at the vitreo-macular interface. Brem et al report that integrin subunits α2, α3 and β1 are present in the retinal inner limiting membrane. As the principle mechanotransducer, integrin α2 and β1 subunit have been shown to induce an enhanced tyrosine phosphorylation under mechanical stretching, which leads to activation of MAP kinase. As the aforementioned suggests, it is conceivable that Müller cells can be activated by mechanical stretching and start to proliferate.
Nature and origin of the glial cells in epiretinal membrane (ERM) associated with idiopathic macular hole (MH) and vitreo-macular traction syndrome (VMS)

The mechanosensitivity of Müller cells may have a significant role in the pathogenesis of idiopathic MH and VMS. During the process of ERM formation, activation of Müller cells could be triggered by an incomplete PVD at the fovea as is seen in the early stages of macular hole formation. It has generally been accepted that anteroposterior and dynamic vitreo-macular traction in the presence of a perifoveal PVD is the primary cause of idiopathic macular hole formation. Bringmann et al suggested that the antero-posterior vitreo-foveal traction could activate Müller cells and result in their subsequent migration and proliferation. Mechanical traction forces exerted onto the Müller cells may increase influx of Ca$^{2+}$ into the cells through stretch-activated channels, which in its turn may result in the activation of Ca$^{2+}$-dependent potassium channels. Such activation may lead to hypertrophic changes including swelling of the cellular processes and enlargement of the cell body. These morphological changes can be seen in clinical optical coherence tomography as intra-retinal cystoid changes and are highly correlated to a partial PVD with vitreo-macular adhesion at the beginning of macular hole formation. The swelling and hypertrophy of Müller cells may induce tissue weakening at the macular area. With persistent traction, a macular tear can occur. Subsequently, persistent vitreo-retinal traction and the injured retina may proceed to stimulate the proliferation, migration and differentiation of Müller cells and the formation of a gliotic scar in stage II macular holes. Schumann et al. in a recent immuno-cytochemical study, identified cells in the MH-associated ERM co-expressing GFAP and CRALBP. From this they concluded that cell migration and proliferation occur early in the course of the disease and that Müller cells are an important component of MH-associated epiretinal cell proliferation. In a clinical observation on the natural history of MH-associated ERM, Cheng et al found an
increased prevalence of ERM in late stage macular hole cases compared to that in early stage MH. In the course of time, MH associated ERMs mature and stabilize.\textsuperscript{28} The self-limiting nature of these ERMs could be partially due to the formation of a complete PVD and thus a relief of the vitreo-macular traction, thereby removing traction stimuli on Müller cells.

**The influence of transforming growth factor-β during the formation of epiretinal membrane associated with vitreoretinal diseases**

Activation of latent TGF-β residing in the ECM, is probably an important initial factor in ERM formation associated with prolonged vitreo-macular traction. Possible triggers of TGF-β activation are known in some diseases, such as, tissue damage (in idiopathic macular hole, peripheral retinal break and rhegmatogenous retinal detachment), breakdown of the blood-retinal barrier (retinal vein occlusion and diabetic retinopathy). However, it has not been clarified in certain diseases such as idiopathic epiretinal membrane (iERM) and vitreo-macular traction syndrome. Therefore, we hypothesize that the traction exerted by a prolonged partial PVD can be sensed by Müller cells at the macula and can trigger the activation of latent TGF-β present in the ECM and the production of TGF-β and other cytokines by the activated Müller cells. This will result in the proliferation of the epiretinal cells and an up-regulation of α-SMA stress fibers in Müller cells (Chapters 5 and 6 of this thesis), which will increase the contractile activity of these cells. The ensuing tissue contraction may further contribute to TGF-β activation, thus further promoting the fibrotic process.

In summary, remodelling of ECM components at the vitreoretinal interface is important during the pathogenesis of vitreoretinal diseases. An understanding of
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des factors may eventually lead to the development of effective and non-surgical approaches to treat and prevent vitreoretinal diseases.
References


