Abnormalities in Vessel Formation in a Mouse Model of Timp3 Deficiency


Bernhard H. F. Weber, Andreas Janssen, Heinrich Schrewe, Ernst Tamm, Chr. Albrecht May, Mathias Seeliger

1Institute of Human Genetics, University of Regensburg, Germany; 2Max-Planck Institute for Molecular Genetics, Berlin, Germany; 3Department of Anatomy, University of Regensburg, Germany; 4Institute of Anatomy, University of Dresden, Germany; 5University Eye Clinic, University of Tuebingen, Germany

Sorsby fundus dystrophy (SFD) is an autosomal dominantly inherited degenerative disease of the retina and is characterized by rapid loss of central vision. One of the early signs is the deposition of material in Bruch’s membrane, a five-layered extracellular matrix (ECM) between the retinal pigment epithelium (RPE) and the choriocapillaris. As a hallmark of this condition, there is a high rate of choroidal neovascularization (CNV), which closely resembles the exudative form of age-related macular degeneration (AMD), a prevalent blinding disorder of multifactorial etiology. SFD is caused by mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3). All TIMP3 mutations known so far are predicted to result in unpaired cysteine residues within the C-terminal portion of the protein which likely cause the formation of high-molecular weight complexes due to disulfate bridge formation. However, it is still unknown how this complex formation leads to retinal degeneration and CNV.

TIMP3 is a member of a family of four secreted proteins (TIMP1 to TIMP4). While TIMP 1, 2, and 4 are soluble and diffusible, TIMP3 is unique as it is covalently bound to the ECM. Originally identified as inhibitors of matrix metalloproteinases (MMPs), the TIMPs are now recognized as proteins with multiple functions independent of their MMP inhibitory activities. Specifically, TIMP3 has recently been found to be a potent inhibitor of angiogenesis by competitively blocking the binding of VEGF to its receptor VEGFR2.

In order to better understand the pathogenesis of SFD and hopefully provide new insight into mechanisms involved in CNV formation, our strategy is to generate mouse models carrying distinct types of Timp3 mutations. Using the Cre-loxP system, a knock-in mouse for a Ser156Cys mutation found to cause early signs of SFD in a large Austrian pedigree was established. On a C57BL/6 background there was a mild phenotype which was more pronounced on a CD1 albinos background closely resembling early SFD manifestations. A second knock-in mouse model expressing a Timp3 Ser156Met mutation was found to be embryonic lethal as a homozygous trait, possibly emphasizing a role for Timp3 during embryonic development. Finally, Timp3 deficiency appears to cause multiple phenotypes affecting tissues that normally express Timp3. So far, reports include, for example, air space enlargement in the lung, dilated cardiomyopathy and chronic hepatic inflammation. In the eye, Timp3 knock-out mice on both a C57BL/6 and a CD1 albinos background reveal a striking dilation of choroidal vessels, although without developing a retinal degeneration phenotype.

To analyze the underlying pathway mechanism of the vessel phenotype in Timp3-deficient mice, we investigated the angiogenic response in an aortic ring angiogenesis assay.
In comparison to wild type, the vascular response in Timp3-deficient tissue showed strongly enhanced vessel outgrowth under spontaneous conditions. Addition of recombinant murine Timp3 reduced this outgrowth to a level comparable to wild type. We further show that this response is mediated through the VEGF/VEGFR2 signaling cascade by pretreatment of Timp3-deficient aortic rings with an inhibitor of VEGFR2 (ZM323881) which dramatically reduced vessel outgrowth. Although to a much lesser degree than in knock-out animals, the excessive outgrowth of vessels also occurs in aortic rings from mutant Ser156Cys knock-in mice. This suggests that the Ser156Cys mutation may impair but not abolish proper binding of Timp3 to VEGFR2.

Downstream signaling pathways of VEGFR2 such as ERK1/ERK2 and p38 were investigated. Both ERK1/ERK2 and p38 show higher levels of phosphorylated protein in knock-out and Ser156Cys knock-in mice compared to wild type. Stimulation with recombinant VEGF increased the amount of phosphorylated proteins regardless of mutation status. Interestingly, the p125 FAK kinase showed increased protein levels in the knock-out but significantly reduced protein expression in the Ser156Cys knock-in.

Nakatsu et al. reported a distinct function for VEGF in the regulation of blood vessel diameter. In a fibrin gel bead assay we coated mouse heart endothelial cells (MHEC) on beads which were then imbedded into fibrin gels. In this three-dimensional network, endothelial cells form capillary like structures. Treatment with increasing concentrations of recombinant VEGF resulted in increased diameters of newly formed capillaries. Addition of recombinant Timp3 strongly reduced this vessel dilation. This suggests a regulatory role of Timp3 in vessel dilation via mechanisms similar to vessel outgrowth. Together, Timp3 appears to be actively competing with VEGF for binding to the VEGFR2 receptor. Consequently, lack of Timp3 or dysfunctional mutated Timp3 results in increased VEGF/VEGFR2 signaling leading to enhanced MAPkinase activity and thus to endothelial cell proliferation and migration, eventually resulting in vessel dilation and increased vessel diameter.

References