PLVAP in diabetic retinopathy: A gatekeeper of angiogenesis and vascular permeability

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GENERAL DISCUSSION AND SUMMARY

The aim of the research described in this thesis was to explore the role of plasmalemma vesicle-associated protein (PLVAP) in development and progression of diabetic retinopathy (DR). DR is a multifactorial disease that can progress from minor changes in vascular permeability, into a vision-threatening proliferative retinal disorder (chapter 1). Previous studies have reported increased PLVAP expression in the retinal vasculature of DR patients, which co-localized with increased vascular permeability. Elucidation of the functions of PLVAP in endothelium and its possible implication in the development of DR may help to develop new, more effective therapeutic approaches in the clinic.

In the normal retina, the neural tissues are protected by the inner and outer blood-retinal barrier (BRB). The inner BRB is mainly formed by specialized endothelial cells that express endothelial tight junctions and an array of specific transcellular transport mechanisms. An intact BRB is essential for proper vision and its breakdown plays an important role in the pathology and vision loss in diabetic macular edema (DME). Several factors, such as inflammation, vascular endothelial growth factor (VEGF), systemic hypertension and reactive oxygen species contribute to BRB leakage. Despite intensive research, the molecular mechanisms underlying BRB breakdown in DR are yet not completely understood. Up to date, two main cellular mechanisms have been proposed to cause BRB loss: increased paracellular transport due to changes in inter-endothelial cell tight junction integrity, and increased trans-endothelial transport mediated by caveolae.

In chapter 2, we used different mouse models of DR to study the mechanisms involved in BRB loss. We observed that VEGF in combination with high glucose levels leads to more severe changes in the BRB than merely elevated glucose or VEGF levels alone. Furthermore, we established that the vascular leakage observed in retinal blood vessels was associated with focal angiogenesis and correlated significantly with Plvap gene expression. Even though the sequence of events that lead to DR in the studied mouse models may not be identical to those in diabetic patients, our findings clearly indicate that PLVAP is involved in regulation of BRB permeability and in particular in transcellular transport.

To further validate the role of PLVAP and other proteins previously described to be involved in increased vascular permeability in DR, we developed an in vitro BRB model consisting of primary retinal endothelial cells, pericytes and astrocytes (chapter 3). We showed, by means of this model, that all these cell types are important for BRB integrity and maintenance. To test whether our model reflects the in vivo changes that occur in DR, we challenged the in vitro barrier with VEGF. This resulted in increased BRB permeability, decreased protein levels and altered structure of endothelial junctions, which is in line with previous reports. Taken together, these findings indicate that our
in vitro BRB model is a useful tool for studying potential therapeutic targets for the treatment of DR.

In chapter 4, we used the advantages of the in vitro BRB model to investigate the impact of lipoprotein-associated phospholipase A2 (Lp-PLA2), a marker of vascular inflammation, and its involvement in diabetes-related leakage of the retinal vasculature. Since there is increasing recognition of pro-inflammatory mediators in the etiology of BRB breakdown in DR, we hypothesized that Lp-PLA2 inhibition may prove to be protective. In our study, we have demonstrated that inhibition of Lp-PLA2 limited BRB damage, indicating that Lp-PLA2 may be an interesting target in managing DR.

In chapter 5, we examined the functional role of PLVAP in regulating VEGF-induced BRB permeability. PLVAP has been associated with vascular permeability in non-barrier endothelium and in the retina in DR, but its functional role in BRB loss was unknown. In this chapter, we show that knockdown of PLVAP in endothelial cells results in decreased VEGF-dependent BRB permeability for differently-sized tracers, both in vitro and in vivo. Additionally, we have revealed that PLVAP regulates vascular permeability by modulating VEGFR2 levels in endothelial cells, which suggests that PLVAP is a potential therapeutic target for DME.

Given the fact that VEGF is a potent inducer of angiogenesis, which acts through VEGFR2, we further studied the role of PLVAP in regulating blood vessel formation in chapter 6. We demonstrated that knockdown of PLVAP expression reduces physiological and pathological angiogenesis in the retina in vivo. In agreement with our previous results, we showed that PLVAP inhibition leads to low levels of VEGFR2 in endothelial cells, which explains their reduced response to VEGF stimulation. In contrast, PLVAP overexpression resulted in increased endothelial cell sprouting and higher numbers of tip cells in vitro, indicating a pivotal role of PLVAP in regulating VEGF-induced angiogenesis. In summary, these data reveal a regulatory role of PLVAP in angiogenesis in association with VEGFR2.

Finally, in chapter 7, we review the current knowledge about PLVAP and its functions. Previous studies have suggested that PLVAP may be involved in regulation of the protein passage through endothelium, maintenance of blood composition, leukocyte transmigration and endothelial tubule formation. Our studies have shed light on the previously unknown role of PLVAP as a cofactor for VEGFR2, and thus as a modulator of VEGF-induced endothelial permeability and angiogenesis. However, there are still many open questions left about the fundamental mechanisms underlying the regulatory function of PLVAP in distinct processes such as inflammation and angiogenesis.

In conclusion, the studies described in this thesis help to understand the molecular mechanisms that underlie BRB loss in DR. We have shown in a mouse model that high glucose concentrations together with elevated VEGF levels have the most profound impact on BRB dysfunction. Furthermore, we have provided new insights into the role
of trans-endothelial transport and increased retinal vascular permeability in DR. Last but not least, we have revealed the crucial role of PLVAP in regulating VEGF-induced angiogenesis and BRB permeability. This makes PLVAP an interesting, endothelial cell-specific therapeutic target for DR that could serve as a safer and more selective alternative for currently used anti-VEGF therapies in the clinic.
REFERENCES:


