Seeing the whole picture:

Every vessel counts

On February 7, 2024, Maria Garcia Collado defended her thesis "The role of endoglin in vascular patterning and malformation across diverse vascular beds" at the Dept. of Medical Biochemistry and Biophysics, Karolinska Institute. Her main supervisor was Associate Professor Lars Jakobsson, Karolinska Institute, Dept. of Medical Biochemistry and Biophysics, Division of Vascular Biology, and her co-supervisor was Professor Arne Östman, Karolinska Institute, Dept. of Oncology and Pathology and Division of Research.

© Yuqiong Wang

Maria Garcia Collado
Department of Medical Biochemistry and Biophysics, Karolinska Institute

Functional blood vessels are crucial to support life. The vascular system is a complex network of vessels—arteries, capillaries, and veins—extending throughout our bodies to transport nutrients, waste products, oxygen, and hormones. Defects in this system give rise to numerous diseases, including hereditary hemorrhagic telangiectasia (HHT) (also known as Osler–Weber–Rendu syndrome), which is caused by loss-of-function mutations in either endoglin (ENG) or activin receptor-like kinase 1 (ALK-1). Patients with HHT develop telangiectasias and arteriovenous malformations in various organs, leading to frequent bleeding. Regarding ocular lesions, patients most commonly develop conjunctival telangiectasias and, less frequently, retinal telangiectasias.

This thesis investigated the mechanisms underlying the development of arteriovenous malformations and the role of endoglin in various vascular beds and developmental stages. Additionally, we performed and optimized the imaging of whole mouse eyes to study their multiple vasculatures and their impact on each other.

Results

Combining tissue clearing with light sheet fluorescence microscopy enabled the imaging of whole mouse eyes. By using an endothelial-specific marker (CD31), we could successfully visualize all the vascular beds of the eye, either together or separately, following *in silico* dissection. This approach allowed us to follow the postnatal development of various vasculatures, observing, for example, extensive remodeling of the iris vasculature as the pupillary membrane regresses. Interestingly, the postnatal iris vasculature showed obvious signs of angiogenesis that were not observed in adult stages. We also applied this method to a model of choroidal neovascularization, which allowed lesion imaging at a higher resolution than that of traditional methods such as optical coherence tomography or fluorescent angiography.

Previous studies conducted by our group and others have shown that postnatal deletion of endoglin (*Eng*) leads to arteriovenous malformation and hyper-sprouting of the retinal vasculature. Our recent study revealed that the postnatal deletion of *Eng* also leads to the malformation of the transient hyaloid vessels, which regress during this time. We found that *Eng* is crucial not only during postnatal stages but also during adulthood, as its deletion results in vascular malformations in a distal location (the pubic symphysis in the pelvic bone), ultimately causing high-output heart failure due to reduced vascular resistance. Finally, we performed single-cell RNA sequencing of the retinal vasculature. This allowed us to investigate the consequences of *Eng* deletion on entire endothelial transcriptomes. This single-cell approach has the potential to reveal key regulatory mechanisms of vascular malformation of the eye.

Key points:

- The imaging of the whole intact eye allows the simultaneous visualization of its multiple vasculatures and how they are interconnected, providing detailed information on vessel growth and remodeling.
- During early postnatal development, the iris vasculature undergoes substantial remodeling and presents signs of angiogenesis as the pupillary membrane and hyaloid vessels regress. During the adult stages, pruning was observed, but no angiogenic sprouts were found.
- The loss of endothelial endoglin during early postnatal stages leads to severe vascular malformations of the retinal vasculature as well as the hyaloid vessels.

Conclusion

The imaging of whole cleared eyes by light sheet fluorescence microscopy allowed us to study the multiple eye vasculatures within their context and follow their development (or regression) throughout the postnatal stages. Furthermore, we found that *Eng* is crucial not only for the maintenance of the vasculature during adult stages but also postnatally in the context of the hyaloid vasculature, which is undergoing regression rather than developing at this stage.

Future directions:

- In future studies, we will study how proliferation affects the development of hyaloid malformations and whether the loss of endoglin also alters hyaloid regression.
- We will also explore our single-cell RNA sequencing data further to identify key regulatory mechanisms of vascular malformation.

References

1. Tual-Chalot S, Garcia-Collado M, et al. Loss of Endothelial Endoglin Promotes High-Output Heart Failure Through Peripheral Arteriovenous Shunting Driven by VEGF Signaling. *Circulation Research*. 2020;126:243-257
2. Krimpenfort LT, Garcia-Collado M, et al. Anatomy of the complete mouse eye vasculature explored by light-sheet fluorescence microscopy exposes subvascular-specific remodeling in development and pathology. *Exp. Eye Res*. 2023 Dec:237:109674.
3. Garcia-Collado M, et al. Endoglin LOF-mediated malformation of the hyaloid vasculature of the eye relies on increased endothelial cell size and proliferation. *Manuscript, 2023*
4. Garcia-Collado M, et al. Dissecting mechanisms of HHT-related arteriovenous malformations through single-cell transcriptomics. *Manuscript, 2023*