The Role of Endothelial Kmt2d in Sprouting Angiogenesis

Terry Xie[1, 2], Sandra Sulser Ponce De Leon[2, 3], Sandeep Sreerama[2, 3], Vivian Chen[2, 4], Angie Serrano[2, 3]

The Harker School, 500 Saratoga Avenue, San Jose, CA[1]; Center for Regenerative Medicine (CReM)[2]; Boston University Chobanian and Avedisian School of Medicine[3]; New Trier High School, 385 Winnetka Ave, Winnetka, IL[4]

Introduction

Sprouting angiogenesis, the development of new vessels from pre-existing vasculature, is critical for organ development. This process relies on endothelial tip cells extending filopodia to determine the direction of vessel growth and stalk cells proliferating to elongate the sprout (Fig. 1). In this study, we focus specifically on intersegmental vessels (Se) and the dorsal longitudinal anastomotic vessel (DLAV) in the zebrafish trunk (Fig. 3). Loss of Kmt2d, a histone methyltransferase involved in gene regulation through histone methylation, causes abnormal vessel sprouting in a zebrafish model (Fig. 2).[1] However, the role of Kmt2d on endothelial cells that participate in the process of sprouting angiogenesis remains unexplored.

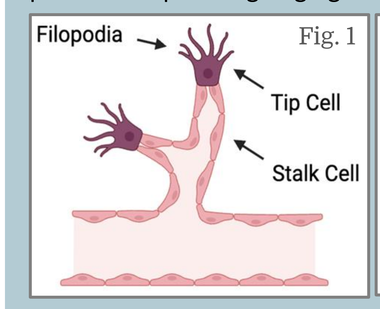


Fig. 1

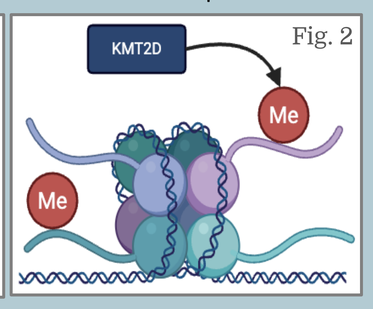


Fig. 2

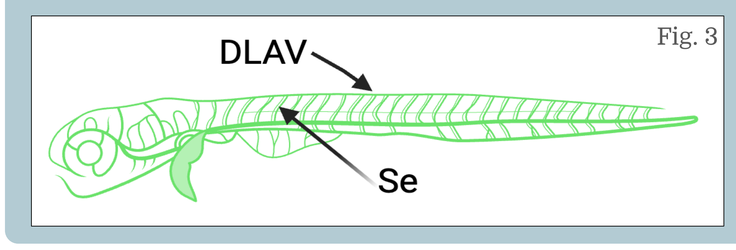
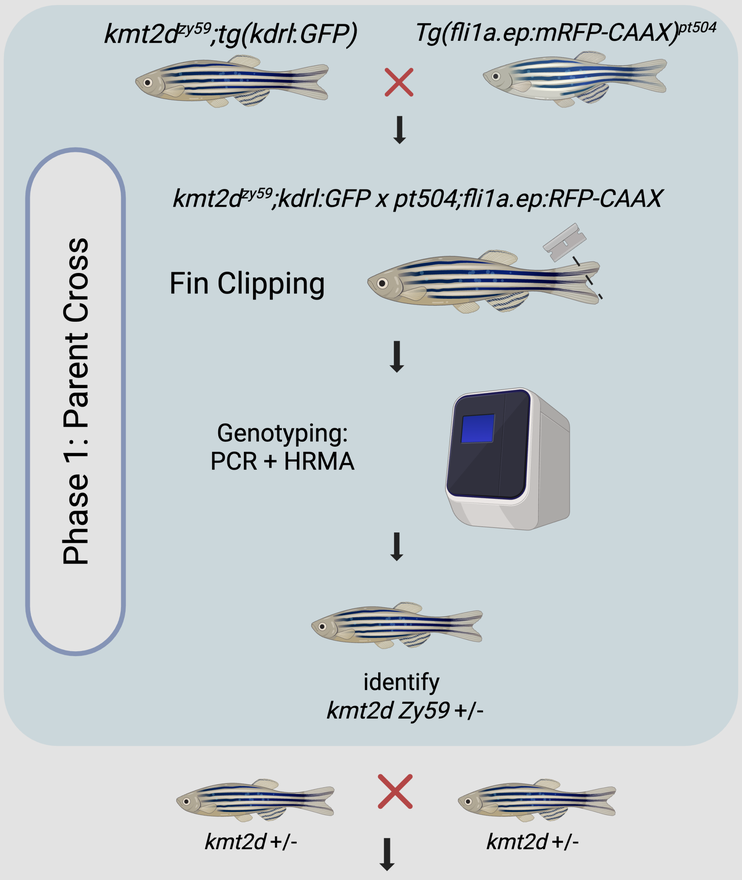


Fig. 3

Research Question

Does loss of Kmt2d affect endothelial cells in vessels established during primary sprouting angiogenesis?

Methodology



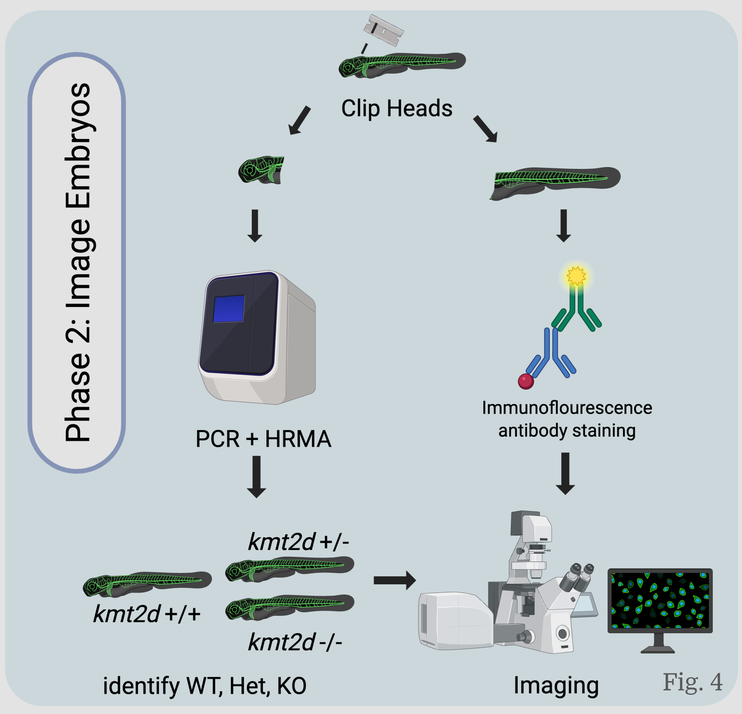
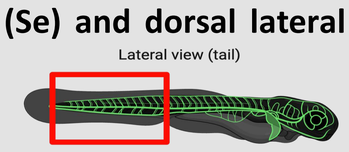


Fig. 4

Results

Fig 5. Zebrafish embryo in brightfield at 72 hours post fertilization (72hpf)

Fig 6. Loss of Kmt2d does not affect gross morphology of intersegmental vessels (Se) and dorsal lateral anastomosis vessels (DLAV) of 72hpf zebrafish embryos



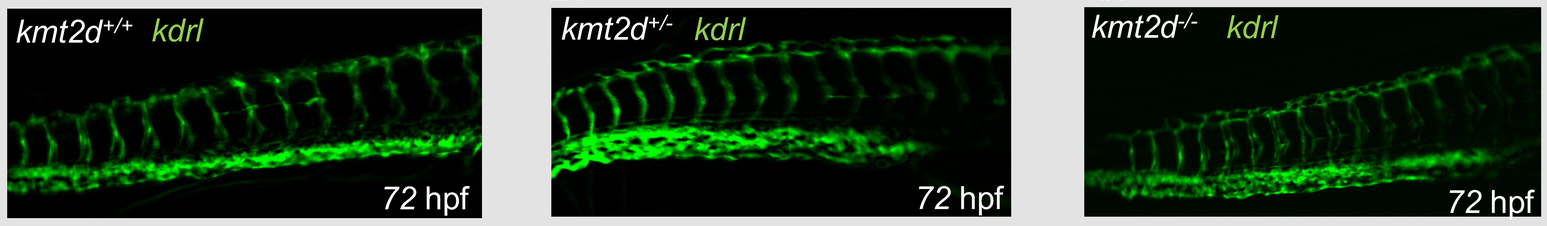


Fig 6. Immunofluorescent images capturing intersegmental vessels (Se) and dorsal lateral anastomosis vessels (DLAV) in the tails of **A)** *wildtype (kmt2d$^{+/+}$),* **B)** *heterozygous (kmt2d$^{-/+}$)* and ***C)*** *knockout (kmt2d$^{-/+}$)* zebrafish embryos expressing GFP in endothelial cells (*kdrl+* cells). Antibody against GFP was used to enhance the signal. n=3 per genotype.

Fig 7. Loss of Kmt2d may lead to smaller Se vessel diameters in 72hpf zebrafish embryos

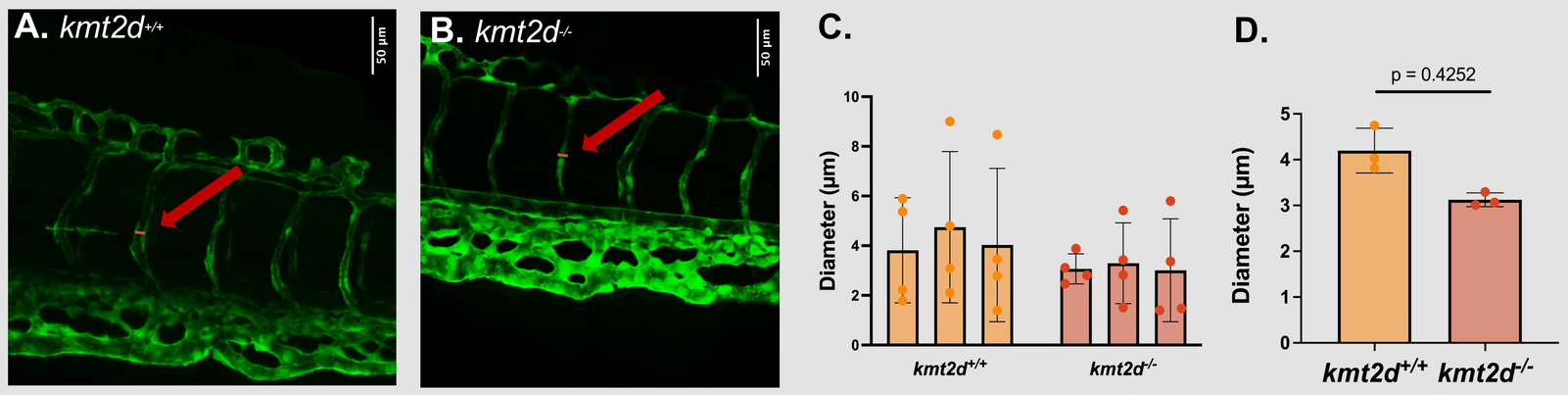


Fig 7. Confocal immunofluorescent images capturing Se vessels in the tails of **A)** *wildtype (kmt2d$^{+/+}$) and* **B)** knockout *(kmt2d$^{-/-}$)* zebrafish embryos at 72 hpf expressing GFP in endothelial cells (*kdrl+* cells) with anti-GFP antibody staining. Images were processed in ImageJ, and diameters of the first 4 Se vessels from each embryo (n=3 per genotype) were measured using the Diameter plugin, as described by Fischer *et al.*[2] **C)** Vessel diameters were calculated by transforming pixel values to micrometers (µm). Each dot corresponds to a vessel diameter value. Error bars represent standard deviation. **D)** Plot of mean vessel diameters for each genotype (n=3). Each dot represents one mean. Nested t-test was performed in all measurements. Error bars represent standard error of the mean. p-value = 0.4252, which is nonsignificant.

Fig 8. Loss of Kmt2d has no significant effect on the number of mitotic endothelial cells in 72hpf zebrafish embryos

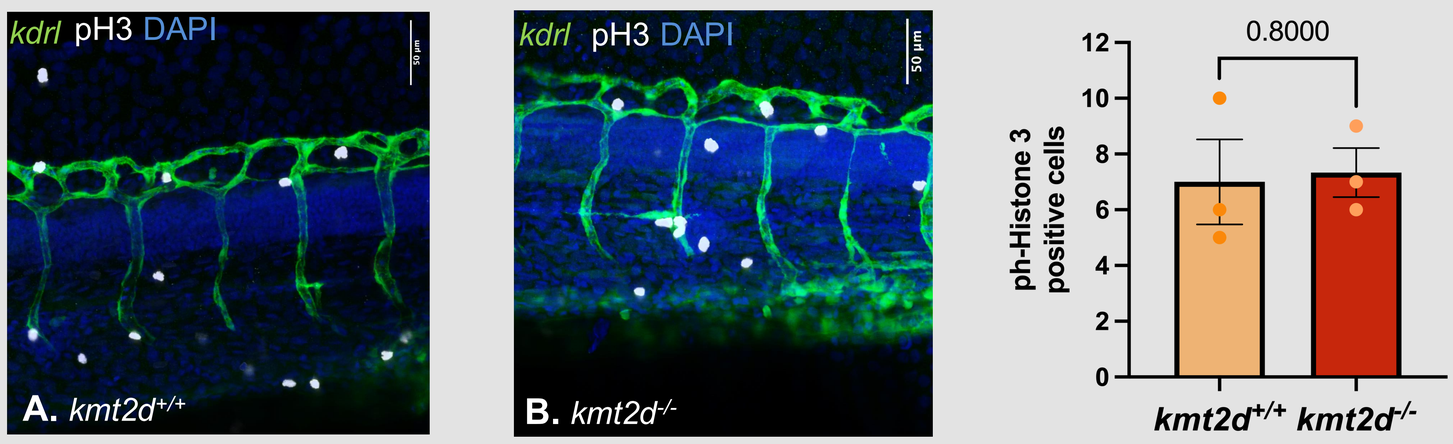


Fig 8. Confocal immunofluorescent images capturing vessels in the tails of **A)** wildtype *(kmt2d$^{+/+}$) and* **B)** knockout *(kmt2d$^{-/-}$)* zebrafish embryos at 72 hpf expressing GFP through *kdrl*+ (Endothelial cell marker) and stained with anti-GFP and anti-PH3 (mitotic cell marker) antibodies, as well as DAPI (DNA marker) dye. Images were processed in ImageJ. **C)** Plot of means number of mitotic endothelial cells for each genotype (n=3). Each dot corresponds to the number of pH3+ cells from a single embryo. A Mann-Whitney Test was performed on all samples, and error bars represent the standard error of the mean. p-value = 0.80, which is nonsignificant.

Conclusions

1. Based on visual inspection of fluorescent microscope images, the overall morphology and development of the Se and DLAV vessels in 72hpf zebrafish embryos was not affected by loss of Kmt2d.
2. Measurement of Se vessel diameters from confocal images revealed a trend of *kmt2d*-null embryos having thinner vessels, which was further backed by visual inspection. This suggests that Kmt2d may play a role in establishing blood vessel diameter. Although these results were not statistically significant, the high variability of our data indicates the need for more biological replicates.
3. Aligning with previous findings, quantification of pH3+ cells from confocal immunofluorescent zebrafish images shows no significant difference between the number of mitotic endothelial cells (representative of endothelial stalk cells) in wild type and knockout embryos, suggesting that loss of Kmt2d does not affect the proliferation of endothelial stalk cells at 72hpf.[1]

References

1. Serrano, M. de et al. (2019) 'Inhibition of notch signaling rescues cardiovascular development in kabuki syndrome', PLOS Biology, 17(9). doi:10.1371/journal.pbio.3000087.
2. Fischer, M.J.M., Uchida, S. and Messlinger, K. (2010) 'Measurement of meningeal blood vessel diameter in vivo with a plug-in for imagej', Microvascular Research, 80(2), pp. 258–266.doi:10.1016/j.mvr.2010.04.004.
3. Eberlein, J. et al. (2021) 'Molecular and cellular mechanisms of vascular development in zebrafish', Life, 11(10), p. 1088. doi:10.3390/life11101088.

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