The Role of KMT2D in Pericyte Development

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Introduction

Kabuki syndrome type 1 (KS1) is a congenital, multisystem disorder associated with variants in the histone methyltransferase Kmt2d. Zebrafish models for KS1 exhibited **impaired angiogenesis** and vascular development as one of the leading causes of cardiovascular defects. One of the two cell types involved in angiogenesis are **pericytes**, which are perivascular cells that originate from the mesoderm or neuroectoderm and wrap around capillaries. In our null Kmt2d zebrafish model, pericyte-associated genes were **downregulated** in a dose-dependent manner. There is a current gap in knowledge regarding how loss of Kmt2d affects pericyte development.

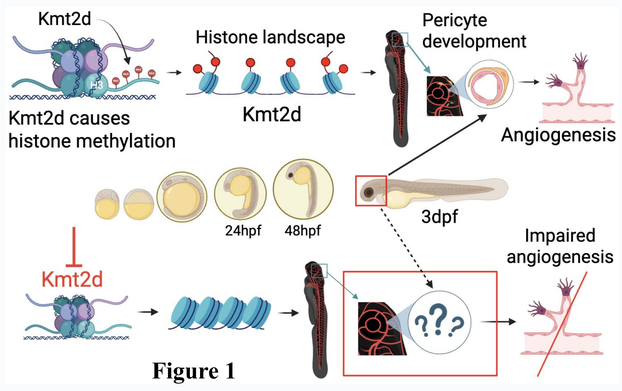
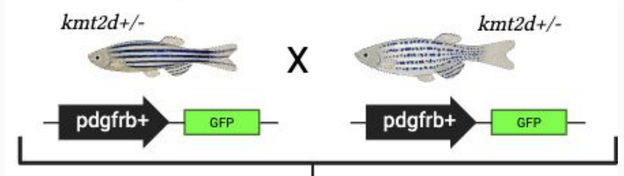


Figure 1

Aim: Test the hypothesis that Km2td supports pericyte development

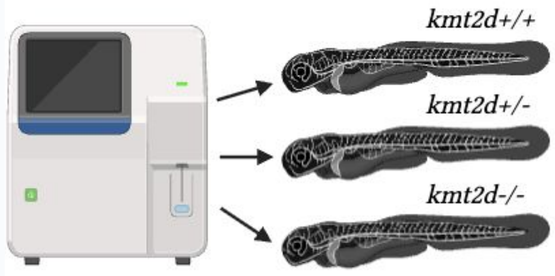
Methods

Bred *heterozygous* Kmt2d adult zebrafish with a pericyte-labeling transgene

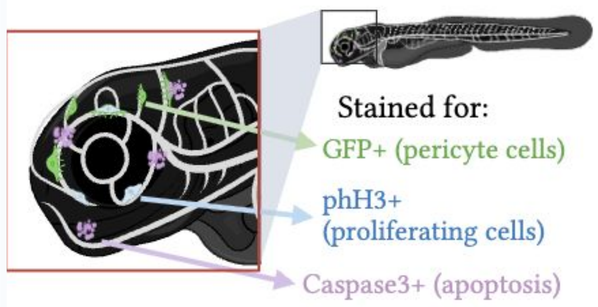


3 days

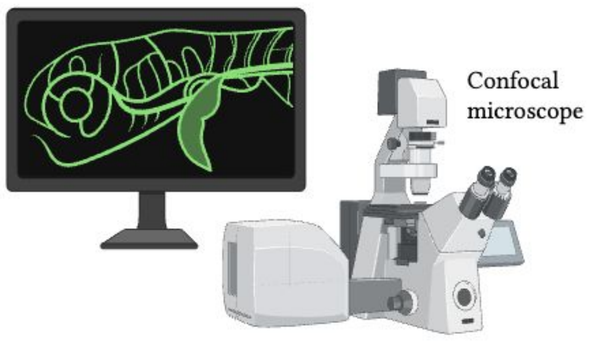
Screened embryos for GFP+ and genotyped via HRMA



Performed immunofluorescence



Imaged cranial vasculature



Blinded image analysis in ImageJ to measure:

- number of pericytes across brain regions
- number of pericytes in proliferation or apoptosis

Figure 2

Results

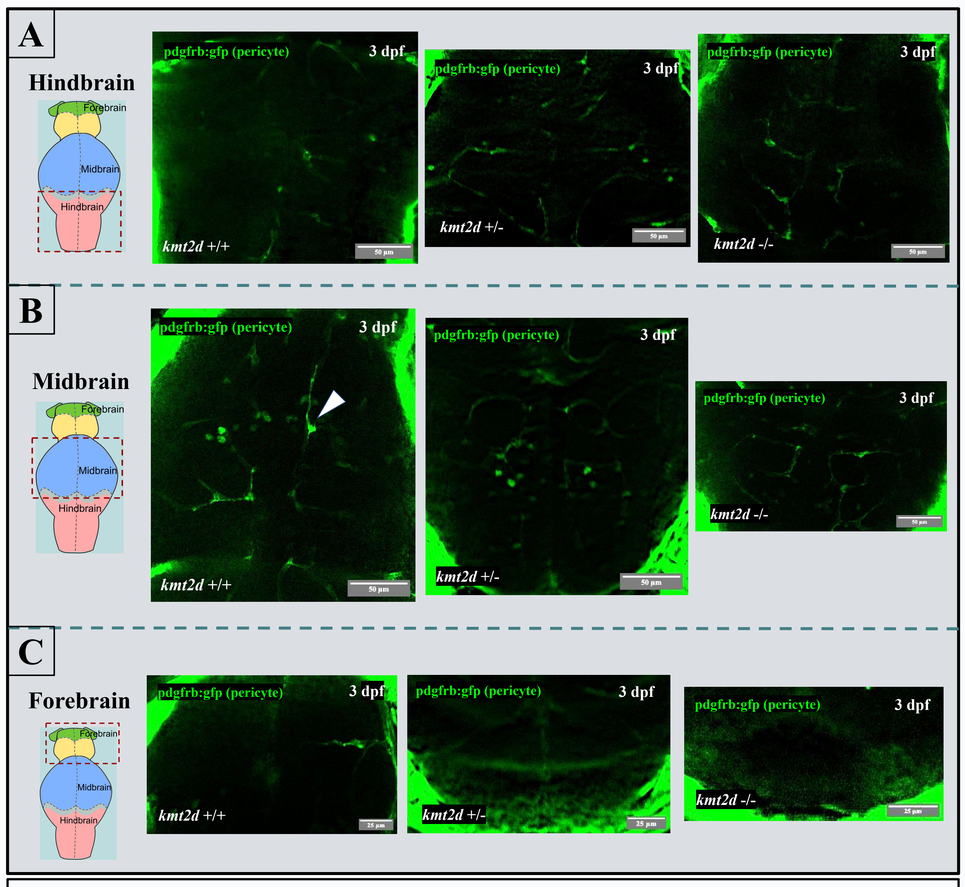


Figure 3: Pericyte numbers decrease with loss of Kmt2d in a dose-dependent manner in the midbrain. Representative immunofluorescence images depicting a dorsal view of wildtype, heterozygous, and null kmt2d zebrafish hindbrain (A) , midbrain (B), and forebrain (C) 3 days post fertilization. Pdgfrb driven GFP labeled in green to mark pericytes.

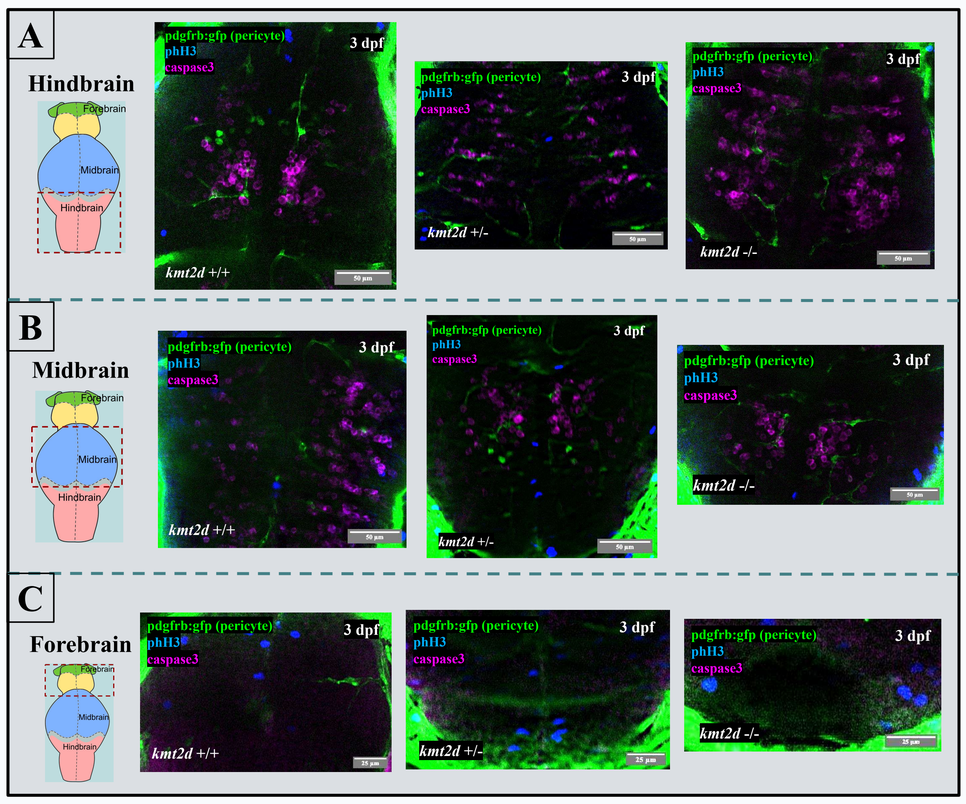


Figure 4: Pericyte proliferation and apoptosis do not appear to be regulated by Kmt2d. Representative immunofluorescence images depicting a dorsal view of wildtype, heterozygous, and null *kmt2d* zebrafish hindbrain (A), midbrain (B), and forebrain (C) 3 days post fertilization. Pdgfrb driven GFP labeled in green to mark pericytes, phH3+ labeled in blue to identify cells in mitosis, and Caspase3+ labeled in purple to show cells in apoptosis.

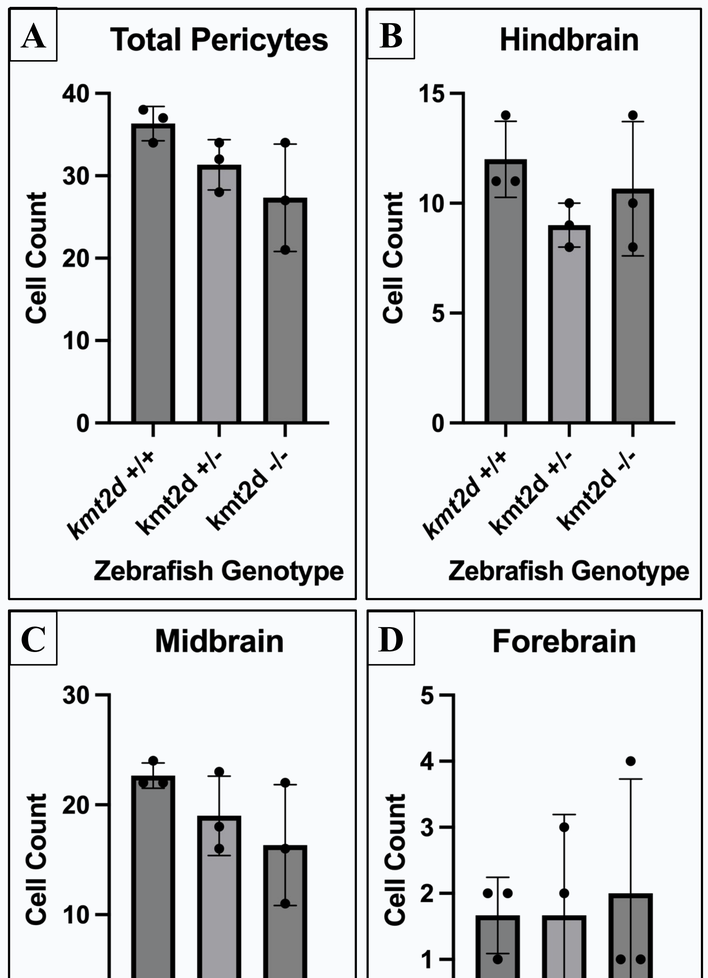


Figure 5: Total pericyte numbers trend towards a dose-dependent decrease with Kmt2d loss. Bar graphs depicting mean cell counts of wildtype, heterozygous, and null *kmt2d* zebrafish pericytes in total (A), and separated by hindbrain (B), midbrain (C), and forebrain (D). Also showing the number of proliferating (E) and apoptotic (F) zebrafish pericytes across genotypes. Error bars denote standard deviations. No statistical significance was determined by a one-way ANOVA.

Discussion

- While not statistically significant, there was a Kmt2d dose-dependent trend of decreasing brain pericytes, especially in the midbrain. This suggests that loss of Kmt2d impairs pericyte development, resulting in a reduced total amount of brain pericytes.
  - Prior studies have shown that loss of pericytes impacts angiogenesis and vasculature. Therefore, the trend of decreasing brain pericyte number may contribute to the vascular phenotypes observed in *kmt2d* mutant zebrafish.
  - Zebrafish are highly phenotypically variable. Thus, future studies to determine the role of Kmt2d in pericyte development will require a larger sample size to make accurate conclusions.
- There was no statistically significant difference nor notable trend in the number of proliferating or apoptotic brain pericytes with loss of Kmt2d. This suggests that Kmt2d may cause a decrease in pericyte number through other mechanisms.

References

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Acknowledgements

We would like to thank Dr. Serrano and the BU RISE program for this opportunity and guidance on this study. We would also like to acknowledge the CReM for the resources provided and the Lawson group for a zebrafish line. Lastly, I would like to thank my family for their unwavering support.