The transcription factor Nfe2l2a is required for development of hematopoietic stem cells in the zebrafish embryo

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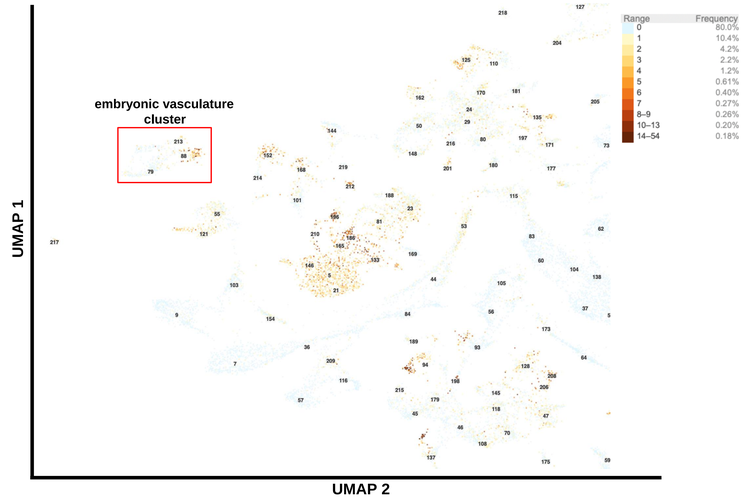
Abstract

Hematopoietic Stem Cells (HSCs) are the self-renewing population of cells that generate all erythrocytes and leukocytes over the lifetime of a vertebrate organism. HSCs are also the therapeutic units of curative bone marrow transplants used in the treatment of blood malignancies and in gene therapy for genetic blood disorders. In all vertebrate embryos, HSCs originate from the floor of the embryonic dorsal aorta during the endothelial to hematopoietic transition. Nascent HSCs will bud into the blood vessel and be carried to maturation sites by the embryonic blood flow. Despite the curative potential of HSC transplants in blood disorders, this approach is limited by low numbers of immunologically compatible HSCs for transplantation. A major objective is to generate unlimited numbers of patient matched HSCs from patient derived induced pluripotent stem cells (iPSCs) to alleviate this challenge to therapy, however it has not yet been possible to generate HSCs from iPSCs. This is likely because key developmental signals remain unknown. This makes the study of HSC development in the vertebrate embryo a key area of biomedical study. Previous work has suggested glucose metabolism generates reactive oxygen species (ROS) which are needed for HSC development. Nfe2l2a is a transcription factor and master regulator of the cellular antioxidant response and is activated by increased levels of ROS in the cell. *Nfe2l2a* has been previously indicated as a regulator of ROS mediated cytokine signaling in adult HSCs. Additionally, *nfe2l2a* is expressed in the embryonic vasculature of zebrafish embryos, which is the origin of HSCs. We sought to determine whether *nfe2l2a* is required for the development of HSCs in the zebrafish embryo. The zebrafish pre-clinical animal model serves as an appropriate model due to evolutionary conservation of hematopoiesis between the zebrafish and humans. We examined the expression of the conserved hematopoietic markers *runx1*, *cmyb*, and *rag1* in embryos injected with an antisense morpholino oligonucleotide which disrupts *nfe2l2a* mRNA translation. We observed a significant reduction in the expression of these markers, indicative of impaired HSC development. Since normal patterning of the embryonic dorsal aorta is required for HSC development, the embryonic vasculature of *nfe2l2a* morphants was also examined for expression of key marker genes, which indicated normal patterning of these tissues. Interestingly, examination of *fli1:EGFP* transgenic embryos injected with *nfe2l2a* morpholino revealed arterio-venous malformations which are indicative of defects in the segregation of the dorsal aorta and cardinal vein. Overall, our work has revealed that *nfe2l2a* activity is required for the development of HSCs in the zebrafish embryo, possibly by controlling segregation of the dorsal aorta and cardinal vein blood vessels during development. Future experiments will be aimed at determining how *nfe2l2a* regulates this process.

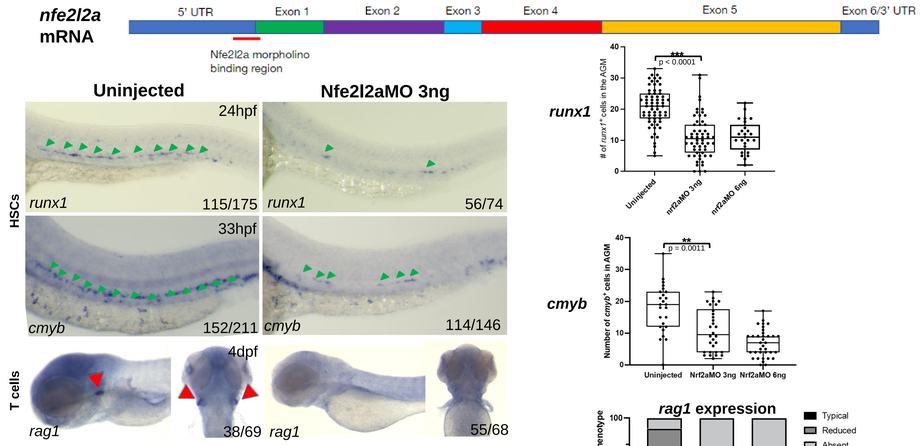
nfe2l2a is expressed in the embryonic vasculature



Two-dimensional representation of single cell RNA sequencing (scRNAseq) data from zebrafish embryos at 24hpf. Each dot represents an individual cell. Cells of the embryonic vasculature cluster together based on transcriptional profile (red box). *Nfe2l2a* is expressed in clusters 213 and 88 which are cells belonging to the dorsal aorta endothelium.

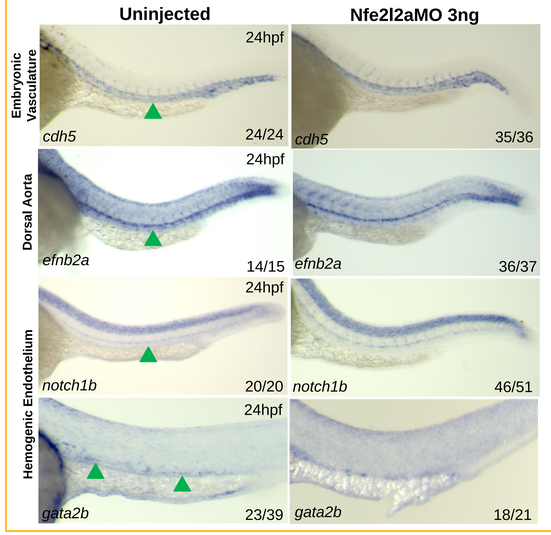
scRNAseq data generated from public dataset by Farnsworth *et al., Developmental Biology,* 2019: http://cells.ucsc.edu

nfe2l2a is required for hematopoietic stem cell development

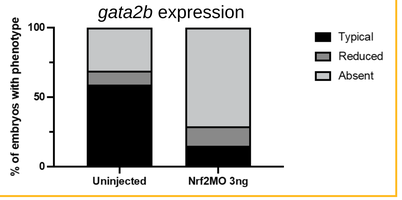


Expression of the HSC marker genes *runx1* and *cmyb* (green arrowheads indicate HSCs) and the T cell marker *rag* in the embryonic thymus (red arrowheads) are significantly reduced in Nfe2laMO embryos. T cells derive directly from HSCs by 4dpf and are an additional indicator of defects in HSC development.

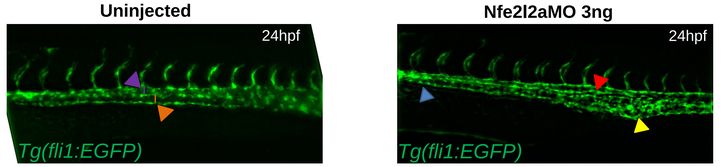
Hemogenic endothelium patterning is defective in *nfe2l2a* knockdown



cdh5, efnb2a, notch1b, and *gata2b* are markers of the embryonic vasculature and the hemogenic endothelium in the developing zebrafish embryo. Expression of *cdh5, efnb2a* and *notch1b* in Nfe2l2aMO was similar to that of these markers in uninjected embryos indicating that the embryonic vasculature was normally patterned. HSCs originate from specialized endothelial cells in the floor of the dorsal aorta called hemogenic endothelium. These cells are marked by the expression of the marker gene *gata2b.* While other vascular markers are normal in morphant embryos, *gata2b* is reduced indicating possible defects in the development of the hemogenic endothelium, which could explain why HSCs do not form in Nfe2l2aMO injected embryos.

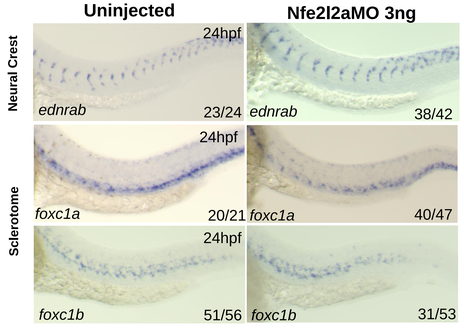


Vascular morphology appears disrupted in *nfe2l2a* knockdown



Tg(fli:EFGP) embryos express EGFP in endothelial cells under the control of the *fli1* promoter. EGFP fluorescence highlights the arterial and venous vasculature in the zebrafish embryo. The separation between the dorsal aorta (DA, purple line and arrowhead) and the cardinal vein (CV, orange line and arrowhead) is clear in uninjected embryos. Nfe2l2a morphants embryos show arterial-venous malformations (red arrowhead), blood vessel fusion (blue arrowhead) and blood vessel dilation (yellow arrowhead). These results indicated morphological defects in embryonic blood vessel development in Nfe2l2a morphant embryos despite normal expression of vascular and arterial marker genes.

HSC developmental niche components are normal in *nfe2l2a* knockdown



HSC developmental niche components are marked by *ednrab* (neural crest cells) and *foxc1a* and *foxc1b* (sclerotome cells). Expression of these markers in Nfe2l2a morphants is similar to that of uninjected embryos suggesting that defects in HSC development are not a result of defects in the assembly of the HSC developmental niche environment.

Results Summary

- Expression of *runx1, cmyb,* and *rag1* show that *nfe2l2a* expression is required for HSC development as the knockdown embryos lack expression of these key marker genes.
- Vascular patterning, however, appears normal in Nfe2l2aMO knockdown embryos based on the expression of *cdh5, efnb2a* and *notch1b* marker genes.
- Development of the hemogenic endothelium might be disrupted in Nfe2l2a morphant embryos as indicated by a reduction in the expression of *gata2b* in the dorsal aorta.
- Despite normal vascular patterning, examination of *fli1:EGFP* transgenic embryos has revealed defects in vascular morphology in Nfe2l2a morphants indicating aspects of vascular development is perturbed.
- HSC developmental niche components appear to assembly normally in Nfe2l2a morphant embryos.
- *Nfe2l2a* could be required for normal development of the hemogenic endothelium and thus HSCs.

Acknowledgements

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