Elucidating new genes involved in hematopoietic specification using a forward genetic screen

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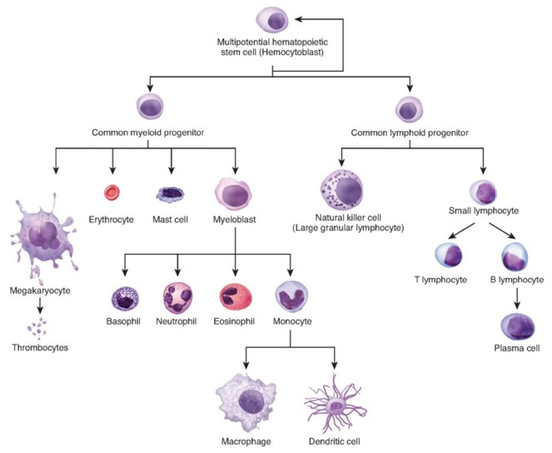
Abstract

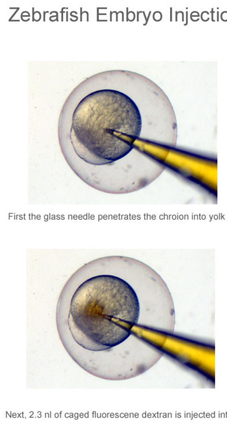
The purpose of this study was to evaluate the specific effects of the *supt16h* gene in Zebrafish on notch signaling and the production of hematopoietic stem cells (HSCs). HSCs are special blood cells that reside at the top of all major blood cell lineages. They are capable of self-renewal, produce cells that differentiate into all types of blood cells, and are critical to homeostatic regulation. Found primarily in bone marrow, HSCs are critical to the proper function of the immune system. By identifying particular genes that control the production of HSCs, we hope to gain a more thorough understanding of how stem cells arise within zebrafish embryos. Should this succeed, our lab would be able to influence the growth of induced pluripotent stem cells (iPSCs) into mature hematopoietic cells capable of producing differentiable cells useful for clinical treatment.

Function of HSCs



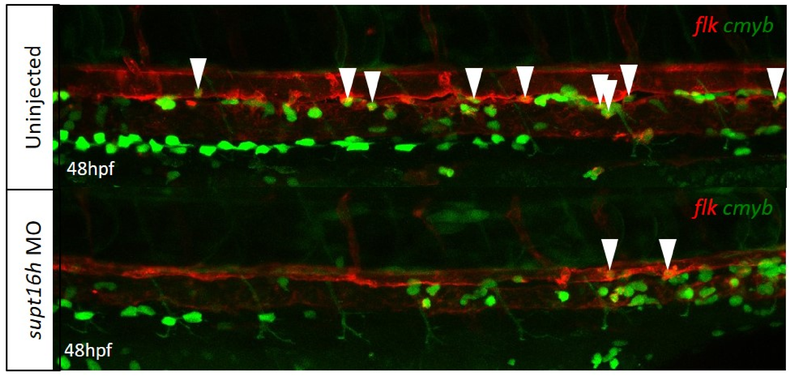
- Capable of self-renewal and the production of the full set of mature blood lineages in the body
- Regulation of apoptosis, the process of programmed destruction of cells that are detrimental or no longer useful to the body
- Can mobilize out of the bone marrow into circulation

Morpholino injection



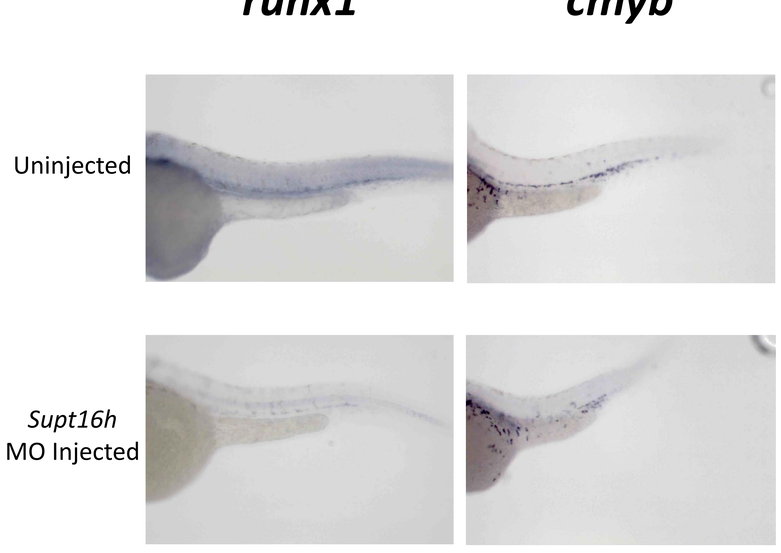
Following the fertilization of the zebrafish eggs, the embryos are harvested and injected with *supt16h* morpholino, which knocks down the expression of the gene in the injected specimens. Uninjected embryos are kept to be used as controls. Injection of the morpholino at such a young stage in development prevents the transcription of the *supt16h* gene in the growing embryos, effectively knocking down the gene. Later on, the embryos were stained with either *runx1* or *cmyb* probes, and in both instances the morpholino injected specimen showed a reduction in the expression of the stained HSC regions. This genetic knockdown was used to observe the effect that the gene under study, *supt16h*, had on two genes (*runx1* and *cmyb*) known to be correlated with the expression of hematopoietic specification.

Supt16h MO knockdown shows reduction in HSCs



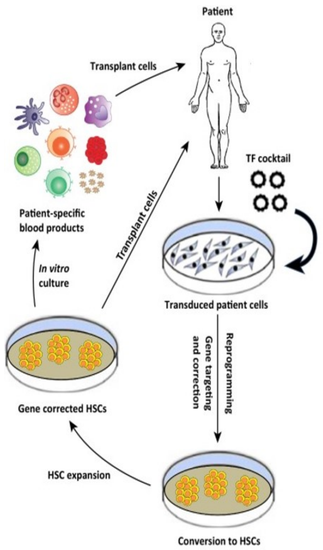
This transgenic line exhibits double positive flk:mcherry and cmyb:gfp cell stains for HSCs. Red staining indicates the vascular system; green stains distinguish HSPC (hemotopoietic stem/progenitor cells). By examining the morpholino injected embryos, it becomes evident that the number of double positive cells is far less than the uninjected sample, thus indicating a decrease in the amount of HSCs present.

Zebrafish *in situ* hybridization samples



- Whole mount *in situ* hybridization with *runx1* probe on embryos at 28hpf
- Whole mount *in situ* hybridization with *cmyb* probe on embryos at 48 hpf
- Reduced amount of HSC staining confirms the hypothesis that *supt16h* has an effect on the expression of two genes known to be involved in HSC expression

Clinical application



The primary clinical application for HSCs is the treatment of leukemia and congenital blood disorders such as aplastic anemia. In patients who experience a reduction in healthy blood cells, the transplantation of HSCs would be an effective method of immediately increasing the production of lymphocytes and other cells necessary for homeostatic regulation. Due to the difficulty of the current method of direct bone marrow transplantation from donor to recipient, scientists have been investigating the use of induced pluripotent stem cells (iPSCs) to be used as candidates for HSC transplantation. By studying the notch signaling and gene expression involved in HSC production in Zebrafish, our lab hopes to understand the *in vivo* production of HSCs in order to synthesis the same cells *ex vivo* in an artificial environment. The ability to produce fully functional HSCs from iPSCs would significantly simplify the ordeal of obtaining viable stem cells to be used to treat victims of blood disease.

Discussion and Conclusions

- After observing both the transgenic line and the *runx1* and *cmyb* stained embryos, it is evident that the *supt16h* gene in Zebrafish plays a role in the development of hematopoietic stem cells. The suppression of the gene caused an obvious decline in the number of HSCs present in the embryos.
- Medical application of iPSCs differentiated into HSCs is a promising treatment for victims of blood diseases to help regenerate cells lost due to a weak immune system
- Further investigation into the mechanism with which *supt16h* functions in the transcription of mRNA is needed to determine whether the gene functions spatially or temporal during the early developmental stages

References

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