**New approaches to bioinformatics analysis in Leiden University Medical Center: bone marrow transplant in Thalasemia patients.**

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1. Introduction

Thalassemia is a genetic blood disorder characterized by impaired hemoglobin synthesis due to mutations affecting globin chain production. Management of the disease relies primarily on life-long blood transfusions and iron chelation therapy, procedures that considerably improve survival but are associated with severe complications, including iron overload, endocrine dysfunction, organ failure, and reduced quality of life. Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only potentially curative option. However, despite significant progress, in thalassemia patients undergoing HSCT the rates of graft rejection and graft-versus-host disease (GvHD) are much higher than in other diseases requiring bone marrow transplantation. To improve outcomes, a deeper understanding of the molecular and cellular mechanisms governing donor–host interactions is required.

1. Description of the problem

The major challenge in HSCT for thalassemia is the hostile host bone marrow environment, which may prevent durable donor cell engraftment. In post-transplant samples, bone marrow shows a mixed cellular composition, with cells originating from both donor and recipient, and distinguishing their origin is technically demanding. Bulk assays cannot resolve this complexity, so specialized computational methods are required. Tools such as Vireo, which use natural genetic variation to assign cells to donor or host in single-cell RNA sequencing data, enable precise identification of cellular origin. Such integrative approaches, combining clinical insight with bioinformatics, are crucial for uncovering molecular determinants of graft success that go beyond standard HLA matching.

1. Related work

Previous studies have highlighted the impaired function of mesenchymal stromal cells in β-thalassemia, including reduced clonogenicity, defective differentiation, and altered secretion of key hematopoietic support factors. These abnormalities, often driven by iron overload and oxidative stress, compromise the bone marrow niche and may hinder donor stem cell engraftment. Although single-cell RNA sequencing has been successfully applied to characterize hematopoietic and immune heterogeneity in other contexts, its systematic use in thalassemia remains limited, and disease-specific microenvironmental mechanisms of transplant failure are still poorly understood.

1. Solution to the problem

We applied scRNA-seq to bone marrow samples obtained from healthy donors and thalassemia patients both before and after HSCT. Using e bioinformatics pipelines (Seurat, SingleCellExperiment) and visualization platforms (CellxGene), we were able to deconvolute the mixed cellular composition of post-transplant samples, accurately tracing the origin of cells and distinguishing donor- from recipient-derived populations. This approach enabled us to identify transcriptional differences between donor and patient cells, particularly at the late stages of erythropoiesis, where donor cells appeared to adopt stress-related or dysfunctional programs under the influence of residual thalassemic host cells and altered niche signals. Such interactions may play a central role in impaired engraftment and graft failure.

To strengthen these observations, additional strategies can be employed, including integration of scRNA-seq with genotyping data to improve donor–recipient cell assignment, the use of trajectory inference methods to capture dynamic lineage transitions, and cross-validation with proteomic or spatial transcriptomic data to map donor–host interactions within the bone marrow microenvironment.

1. Conclusions and future work

Our findings indicate that host–donor interactions within the thalassemic bone marrow niche can shape HSCT outcomes. Identified genetic and antigenic markers could be incorporated into extended donor–recipient matching protocols to reduce graft rejection rates.

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References

1. Li, Z., Wang, X., Zhang, H., & Xu, Y. (2024). Exploring the bone marrow microenvironment in β-thalassemia using single-cell sequencing approaches. Frontiers in Immunology, 15, 1403458. doi:10.3389/fimmu.2024.1403458
2. Hua, P., Roy, N., De La Fuente, J., Wang, G., Thongjuea, S., Clark, K., Roy, A., Psaila, B., Ashley, N., Harrington, Y., Nerlov, C., Watt, S., Roberts, I., & Davies, J. (2019). Single-cell analysis of bone marrow-derived CD34+ cells from children with sickle cell disease and thalassemia. Blood, 134(23), 2111–2115. doi:10.1182/blood.2019002301
3. Locatelli, F., Corbacioglu, S., Lang, P., et al. (2024). Exagamglogene autotemcel for transfusion-dependent β-thalassemia. New England Journal of Medicine, 391(6), 515–524. doi:10.1056/NEJMoa2309676
4. Triana, S., et al. (2021). Single-cell proteo-genomic reference maps of the human blood and bone marrow. Nature Immunology, 22(12), 1577–1589. doi:10.1038/s41590-021-01059-0
5. Zhang, Y., et al. (2022). Temporal molecular program of human hematopoietic stem and progenitor cell development. Developmental Cell, 57(1), 1–16.e6. doi:10.1016/j.devcel.2022.07.019
6. Huang, Y., McCarthy, D. J., & Stegle, O. (2019). Vireo: Bayesian demultiplexing of pooled single-cell RNA-seq data without genotype reference. Genome Biology, 20(1), 273. doi:10.1186/s13059-019-1865-2
7. Nassiri, I., McCarthy, D. J., & Stegle, O. (2024). Evaluation of genetic demultiplexing of single-cell RNA-seq data using Vireo. Bioinformatics Advances, 4(1), vbae085. doi:10.1093/bioadv/vbae085