Identification of co-expression modules associated with the enrichment of Long-Term Hematopoietic Stem Cells (LT-HSCs)

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Introduction

LT-HSCs are rare cells capable of self-renewal and long-term reconstitution of all hematopoietic lineages, playing a key role in transplantation and gene therapy. Despite advances in ex vivo expansion protocols, identifying and quantifying functional LT-HSCs after culture remains a major challenge. Surface markers often lose specificity under culture-induced changes, making xenotransplantation the gold standard for functional validation. This study proposes the use of gene and protein co-expression analyses to identify more robust and stable markers associated with LT-HSC enrichment.

Methods

$CD34^+$ cells mobilized from peripheral blood (4 biological replicates) were cultured for seven days in StemSpan SFEM II medium supplemented with cytokines and various combinations including the small molecule UM171. Cell viability and proliferation were monitored on days 2, 5, and 7. Subsequently, FACS sorting was performed to isolate populations enriched for LT-HSCs based on CD34, CD45RA, CD90, and EPCR expression. Samples were constructed with different proportions of the $CD34^+CD45RA^-CD90^+EPCR^+$ immunophenotype (0%, 5%, 10%, 20%, and 50%). In parallel, fresh $CD34^+$ cells (also 4 biological replicates) were sorted based on CD34, CD38, CD45RA, and CD90 expression, generating samples with varying levels of enrichment for the $CD34^+CD38^-CD45RA^-CD90^+$ phenotype (0%, 2.5%, 5%, 10%, and 25%). Total RNA (RIN > 8) was extracted and used to construct bulk RNA sequencing libraries using the Illumina Stranded mRNA Prep kit. For cultured samples, protein content was also extracted and analyzed by LC-MS/MS in collaboration with LNBio-CNPEM. Co-expression analyses will be performed using the WGCNA package in R to identify modules associated with the LT-HSC phenotype.

Results

Extraction and quantification protocols were standardized and validated, enabling the processing of both fresh and cultured samples for transcriptomic and proteomic profiling. Flow cytometry revealed that the UM171-containing culture condition significantly increased the proportions of $CD34^+CD45RA^-$, $CD45RA^-CD90^+$, and $CD90^+EPCR^+$ populations, suggesting greater LT-HSC enrichment. All RNA samples exhibited suitable quality and purity for sequencing. Proteomic analysis identified 5,703 proteins with high consistency across samples.

Discussion

The application of WGCNA will enable the identification of co-expression modules whose eigengenes correlate with LT-HSC enrichment, offering a more comprehensive approach compared to individual gene analysis. Integration of transcriptomic and proteomic datasets will allow the identification of central (hub) genes within modules associated with the LT-HSC phenotype, potentially serving as functional markers under diverse culture conditions.

Conclusion

Gene and protein co-expression network analysis appears to be a promising strategy to overcome the limitations of traditional surface markers, enabling the definition of more stable molecular targets for the identification and functional quantification of LT-HSCs. The data generated may support the development of improved culture protocols and clinical applications involving hematopoietic stem cells.

References

Anjos-Afonso, F., & Bonnet, D. (2023). Human CD34+ hematopoietic stem cell hierarchy: how far are we with its delineation at the most primitive level? *Blood*, *142*(6), 509–518. https://doi.org/10.1182/blood.2022018071

Fares, I., Chagraoui, J., Lehnertz, B., MacRae, T., Mayotte, N., Tomellini, E., Aubert, L., Roux, P. P., & Sauvageau, G. (2017). EPCR expression marks UM171-expanded CD34+ cord blood stem cells. Blood, 129(25), 3344–3351. https://doi.org/10.1182/blood-2016-11-750729

Langfelder, P., & Horvath, S. (2008). WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*. https://doi.org/10.1186/1471-2105-9-559

Notta, F., Doulatov, S., Laurenti, E., Poeppl, A., Jurisica, I., & Dick, J. E. (2011). Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science*, *333*(6039), 218–221. https://doi.org/10.1126/science.1201219

Wilkinson, A. C., Igarashi, K. J., & Nakauchi, H. (2020). Haematopoietic stem cell self-renewal in vivo and ex vivo. *Nature Reviews Genetics*, *21*(9), 541–554. https://doi.org/10.1038/s41576-020-0241-0.