A Transgenic System for Rapid Magnetic Enrichment of Rare Embryonic Cells

Samuel J. Wattus^{1,2} and Leonard I. Zon^{1,2}

Abstract

Collecting large numbers of rare cells for high-throughput molecular analysis remains a technical challenge, primarily due to limitations in existing technologies. In developmental biology this has impeded single-cell analysis of primordial organs, which derive from few cells. In this study, we share novel transgenic lines for rapid cell enrichment from zebrafish embryos using human surface antigens for immunological binding and magnetic sorting. As proof of principle, we tagged, enriched, and performed single-cell RNA sequencing on nascent hematopoietic stem/progenitor cells and endothelial cells from early embryos. Our method is a quick, efficient, and cost-effective approach to a previously intractable problem.

Keywords: magnetic, enrichment, single cell, tool, hematopoiesis

LIMITED ACCESS TO EMBRYONIC TISSUE has long stymied progress in developmental biology. Fundamental questions about the formation of complex organ systems are based on the activities of extremely rare cell populations in the embryo. For the past several decades, zebrafish have emerged as a powerful model system to address these issues. With high numbers of externally fertilized and transparent embryos, zebrafish allow for unparalleled live imaging of early development. Still, the ability to extract and molecularly analyze cells of interest from zebrafish embryos depends upon conventional fluorescence-activated cell sorting (FACS).

Although FACS is a powerful technique capable of cell-type purification, collecting rare cells *en masse* requires hours of sort time, which can introduce variable amounts of cell stress due to fluid shear forces and wait time on ice.¹ This is incompatible with high-throughput microfluidic approaches to single-cell RNA sequencing such as 10X or InDrops, which require high viability and typically necessitate sample preparation in <1 h. Magnetic enrichment of cells circumvents these problems through gentle handling and simultaneous separation of millions of cells at a time.¹ However, commercially available reagents for magnetic enrichment are currently only designed for use with human and mouse cells.

In answer to this, we generated transgenic zebrafish stably expressing truncated forms of human *CD4* (hCD4) and *LNGFR* (hLNGFR) antigens under control of the pan-endothelial *kdrl* promoter² and the hematopoietic stem and progenitor (HSPC) specific *runx1* + 23 enhancer.³ These truncated surface proteins have low homology with the zebrafish proteome and completely lack a

¹Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts, USA.

²Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, Cambridge, Massachusetts, USA.

FIG. 1. (A) A schematic overview of magnetic enrichment. Rare cells, such as HSPCs, express surface peptides that are bound with magnetic beads. The sample is passed over a magnetic column and unlabeled cells are washed off. (B) Flow cytometric analysis of *runx1*+23:*mCherry*; *runx1*+23:*hLNGFR* embryos before and after magnetic enrichment. After MACS enrichment, HSPCs comprised 42% of the sample (right) as compared with an unenriched sample, in which HSPCs only make up 0.22% of live cells (left). (C) Analysis of *kdrl*:GFP; *kdrl*:hCD4 embryos before and after MACS enrichment. After magnetic sorting, endothelial cells were 85% of live cells (right) as compared with an unenriched sample, in which endothelial cells only make up 3.9% of live cells (left). (D) A comparison of the percentage of sequencing reads mapping to the mitochondrial genome in endothelial cells harvested by FACS versus MACS suggests that MACS-enriched cells exhibit lower levels of stress (***) ($p < 0.0001$ unpaired *t*-test). (E) A heatmap of common stress response genes illustrates that MACS-enriched endothelial cells undergo less stress than those isolated by FACS. FACS, fluorescence-activated cell sorting; HSPCs, hematopoietic stem and progenitors; MACS, magnetic-activated cell sorting.

cytoplasmic domain, meaning that they should not interact with endogenous proteins nor initiate any downstream signaling. Instead, they serve as targets that can be bound by antibodies and microbeads designed for use in human systems and can be used to magnetically separate cells of interest⁴ from zebrafish embryos (Fig. 1A). Magnetic enrichment only requires a 20 min incubation step with immunologically conjugated microbeads and 10 min for manual or automated column separation.

As proof of principle, we crossed our existing *runx1*+23:*mCherry* line with the *runx1*+23:*hLNGFR* line, subjected embryos to magnetic enrichment at 48 and 72 h postfertilization (hpf), and analyzed enrichment by flow cytometry. At these stages, HSPCs have just emerged from the dorsal aorta and there are only around ~100 HSPCs per embryo⁵ or ~0.2% of live cells. After magnetic enrichment, this could be massively increased ~100- to 200-fold so that up to 42% of cells in the sample were *runx1*+23:*mCherry*+ (Fig. 1B). Similar results were produced with *kdrl*:*GFP*;*kdrl*:*hCD4* embryos at 24 hpf (Fig. 1C). After enrichment, samples could be run as an enriched population for single-cell approaches with appropriate data filtering, or could be completely purified by a short FACS run. With this approach, we were able to isolate enough *runx1*+23:*mCherry*+ cells in 45 min to do single-cell RNA sequencing with the InDrops platform, which typically requires >20,000 cells to input.⁶ In contrast, we were unable to harvest the requisite number of embryonic *runx1*+23:*mCherry*+ cells by FACS alone within 2 h to encapsulate through InDrops.

However, we were able to undertake comparative isolation and single-cell sequencing with our *kdrl*:*hCD4* and *kdrl*:*GFP* lines by magnetic enrichment with magnetic-activated cell sorting (MACS) and FACS, respectively. Endothelial cells that were magnetically enriched and sequenced had on average fewer reads mapping to the mitochondrial genome than those isolated by FACS, supporting the notion that this method introduces lower levels of cell stress^{7,8} (Fig. 1D). Consistent with this, we also found significantly higher expression of multiple stress response genes in cells isolated by FACS versus those enriched by MACS (Fig. 1E). We expect these antigens can be adapted to other well-characterized promoters and enhancers for isolation of other rare populations. Our modified dissociation protocol for this approach is available online ([dx.doi.org/10.17504/protocols.io.bhquj5ww](https://doi.org/10.17504/protocols.io.bhquj5ww)). Our approach is a powerful tool that complements the ability to visualize activities of rare cells in zebrafish embryos and enables massive enrichment of these populations for molecular analysis.

Disclosure Statement

L.I.Z. is a founder and stockholder of Fate Therapeutics, Marauder Therapeutics, CAMP4 Therapeutics, and Scholar Rock. S.J.W. declares no conflicts of interest.

Funding Information

This work is supported by Howard Hughes Medical Institute, the Edward P. Evans Foundation, and National Institutes of Health grants P01-HL032262, U54-DK110805, R24-DK092760, R24-OD017870, U01-HL134812, P01-HL131477, R01-HL144780-01 to L.I.Z. S.J.W. is supported by a National Institutes of Health predoctoral fellowship 1F31HL149154-01.

References

1. Sutermaister BA, Darling EM. Considerations for high-yield, high-throughput cell enrichment: fluorescence versus magnetic sorting. *Sci Rep* 2019;9:227.
2. Choi J, Dong L, Ahn J, Dao D, Hammerschmidt M, Chen JN. FoxH1 negatively modulates flk1 gene expression and vascular formation in zebrafish. *Dev Biol* 2007;304:735–744.
3. Tamplin OJ, Durand EM, Carr LA, Childs SJ, Hagedorn EJ, Li P, *et al.* Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. *Cell* 2015;160:241–252.
4. Miltenyi S, Muller W, Weichel W, Radbruch A. High gradient magnetic cell separation with MACS. *Cytometry* 1990;11:231–238.

5. Murayama E, Sarris M, Redd M, Le Guyader D, Vivier C, Horsley W, *et al.* NACA deficiency reveals the crucial role of somite-derived stromal cells in haematopoietic niche formation. *Nat Commun* 2015;6:8375.
6. Klein AM, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, *et al.* Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* 2015;161:1187–1201.
7. Ilicic T, Kim JK, Kolodziejczyk AA, Bagger FO, McCarthy DJ, Marioni JC, *et al.* Classification of low quality cells from single-cell RNA-seq data. *Genome Biol* 2016;17:29.
8. Lun AT, McCarthy DJ, Marioni JC. A step-by-step workflow for low-level analysis of single-cell RNA-seq data with Bioconductor. *F1000Res* 2016;5:2122.

Address correspondence to:
Leonard I. Zon, MD
Stem Cell Program
Karp Family Research Building
1 Blackfan Circle
Boston, MA 02115
USA

E-mail: zon@enders.tch.harvard.edu