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PERSPECTIVE

Lightening the way of hematopoiesis: Infrared laser-mediated lineage tracing with high spatial-temporal resolution

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Hematopoiesis refers to the developmental process generating all blood lineages. In vertebrates, there are multiple waves of hematopoiesis, which emerge in distinct anatomic locations at different times and give rise to different blood lineages. In the last decade, numerous lineage-tracing studies have been conducted to investigate the hierarchical structure of the hematopoietic system. Yet, the majority of these lineage-tracing studies are not able to integrate the spatial–temporal information with the developmental potential of hematopoietic cells. With the newly developed infrared laser-evoked gene operator (IR-LEGO) microscope heating system, it is now possible to improve our understanding of hematopoiesis to spatial–temporal-controlled single-cell resolution. Here, we discuss the recent development of the IR-LEGO system and its applications in hematopoietic lineage tracing in vivo.

Hematopoiesis is a vital developmental process in which all blood cells, including erythrocytes and leukocytes, are generated. It has long been recognized that vertebrate hematopoiesis occurs in multiple waves in various anatomic sites. In mammals, the first or primitive wave of hematopoiesis begins in the yolk sac (YS) and generates predominantly erythrocytes and macrophages [1]. Shortly after the primitive hematopoiesis, a transient erythroid/myeloid progenitor (EMP) population is observed in the YS [2,3]. Finally, the definitive wave of hematopoiesis emerges in the aorta–gonad–mesonephros (AGM) region, where the hemogenic endothelium in the ventral wall of dorsal aorta gives rise to hematopoietic stem and progenitor cells (HSPCs) through endothelial-to-hematopoietic transition (EHT). These HSPCs subsequently migrate to the fetal liver for a transit expansion and differentiation and finally reach the bone marrow, where they continuously generate all hematopoietic lineages, except for some tissue resident macrophages, in adulthood [4,5]. In addition to these two conventional waves of hematopoiesis, hematopoietic activities have also been reported in other anatomic sites. For example, definitive hematopoietic activity has been detected in the allantois and chorionic mesoderm [6]; hematopoietic stem cell (HSC) activities have also been found in the placenta, YS, and head region [7–10]. Hence, a comprehensive understanding of the development of hematopoietic cells from different waves of hematopoiesis needs to integrate the information from multiple dimensions: when and where these cells are generated; what kinds of progenies these different waves of hematopoiesis would give rise to; and where the mature progenies derived from different waves of hematopoiesis reside.

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One approach used to dissect the blood cell development of the different waves of hematopoiesis is to collect specific hematopoietic tissues at desired stages and conduct in vitro/ex vivo culture or transplantation assay [5,6] (Figure 1A). However, this strategy provides only relatively low spatial resolution of cell lineage tracing, and moreover, the in vitro/ex vivo culture or transplantation assay may not faithfully recapitulate the in vivo hematopoietic activities. To avoid this problem, cell fate mapping needs to be performed in vivo. The most widely used strategy of in vivo lineage tracing is the hematopoiesis-specific promoter-controlled CreER-loxP fate mapping system (Figure 1B). Because this system depends largely on the specificity of the promoter, it is therefore impossible to reach single-cell resolution and may also lead to inconsistent results and even contradictory conclusions. For example, *Tie2* and *Flt3* promoter-mediated fate mapping suggests that EMPs are the origin of most tissue resident macrophages in adult mice, while *Kit* locus-mediated lineage tracing supports the HSC origin theory [11,12]. Recently, cell barcoding techniques have been utilized for fate mapping studies (Figure 1C). These techniques include Polylox barcoding, CRISPR/Cas9-induced random mutations, and retroviral infection-induced insertions [13–15]. A similar approach, which uses fluorescent proteins covering different spectra to serve as the color "barcoding," has also been developed and employed for fate mapping analysis (Figure 1D). Basically, random loxP recombination leads to various copy numbers of different fluorescent proteins in distinct progenitors. Each progenitor then possesses its unique "color hue" because of the combination of different fluorescent proteins. Because the expression of these fluorescent proteins is controlled by ubiquitously expressed promoters, the progenies can be identified according to the same color hue as their progenitors [16,17]. Compared with other barcoding methods, color "barcoding" offers direct visualization of the progenies in vivo. The resolution of this method depends on the precision with which different color hues are distinguished. In addition, single-cell RNA sequencing data have been applied to reconstruct the differentiation trajectories of hematopoiesis [18,19]. Although these methods are able to achieve single-cell resolution, they cannot efficiently

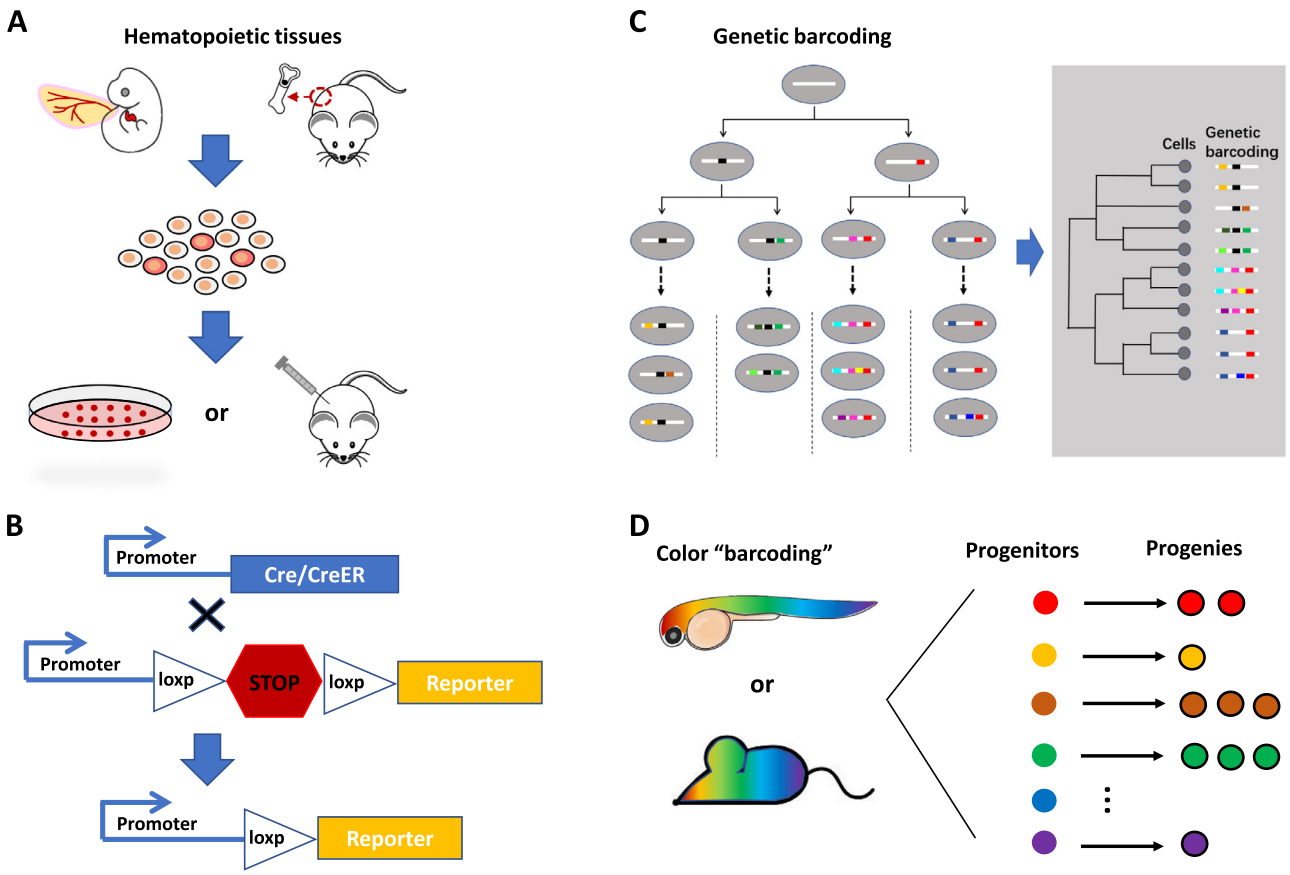

Figure 1. Current lineage tracing techniques applied to hematopoiesis. **(A)** Collection of specific hematopoietic tissues for in vitro/ex vivo culture or transplantation assay. **(B)** Promoter-controlled Cre/CreER-loxP fate mapping system. **(C)** Genetic barcoding strategy (Polylox barcoding, CRISPR/Cas9-induced random mutations or retroviral infection-induced insertions) for lineage tracing. **(D)** Color "barcoding" strategy for lineage tracing.

integrate the spatial–temporal information of hematopoietic progenitors with their differentiation potential.

Since the early 1990s, lasers have been introduced to activate the heat shock response in worm, fly, and zebrafish [20–25]. The high precision of the laser beam makes it possible to activate genes in specific cells at superior resolution. However, these early attempts utilized visible lasers, which require relatively long irradiation time and often have toxic effects on the targeted cells [21,25]. In the past few years, an improved laser system, the infrared laser-evoked gene operator (IR-LEGO) microscope heating system, has been introduced for gene activation and cell fate mapping analysis in *Caenorhabditis elegans* and zebrafish [26–30]. Because of the transparency of the embryos and conserved hematopoietic program [1], zebrafish have recently emerged as an ideal model for hematopoietic fate mapping study with the state-of-art optical techniques. Briefly, a reporter zebrafish line, in which the LoxP–DsRed–Stop–LoxP–GFP cassette is under the control of a hematopoiesis-specific promoter, will be created and outcrossed with the heat-shock promoter-directed CreER transgenic fish, *Tg(hsp70:mCherry-T2a-CreER^{T2})*. With the double-transgenic fish, the IR-LEGO system will utilize an infrared laser to generate local heat to induce the expression of CreER, followed by 4-OH-tamoxifen (4-OHT) treatment. As a result, the heat-shocked progenitor cells and their progenies will be tracked by the expression of GFP. Thus, the IR-LEGO system can genetically label progenitor cells in a particular position at a desired timing. The cell fate of these labeled cells then can be traced. Because this method depends only on the position and timing of laser shining, it exhibits no labeling bias of cell types. With this method, we have suggested the HSC origin of tissue resident macrophages and the existence of non-HSC-derived T cells [26,27,29]. However, the original IR-LEGO technique cannot reach single-cell resolution and hampers further dissection of hematopoietic lineages. To achieve fate mapping with single-cell resolution, we need to (1) confine the heating volume in a single-cell dimension; (2) establish a criterion to determine the optimal heat shock condition for single-cell labeling; and (3) avoid cell damage caused by overheating.

Recently, we advanced the IR-LEGO technique to single-cell labeling resolution [31] (Figure 2). To achieve high efficiency of single-cell labeling, a water-immersion objective with a large numerical aperture

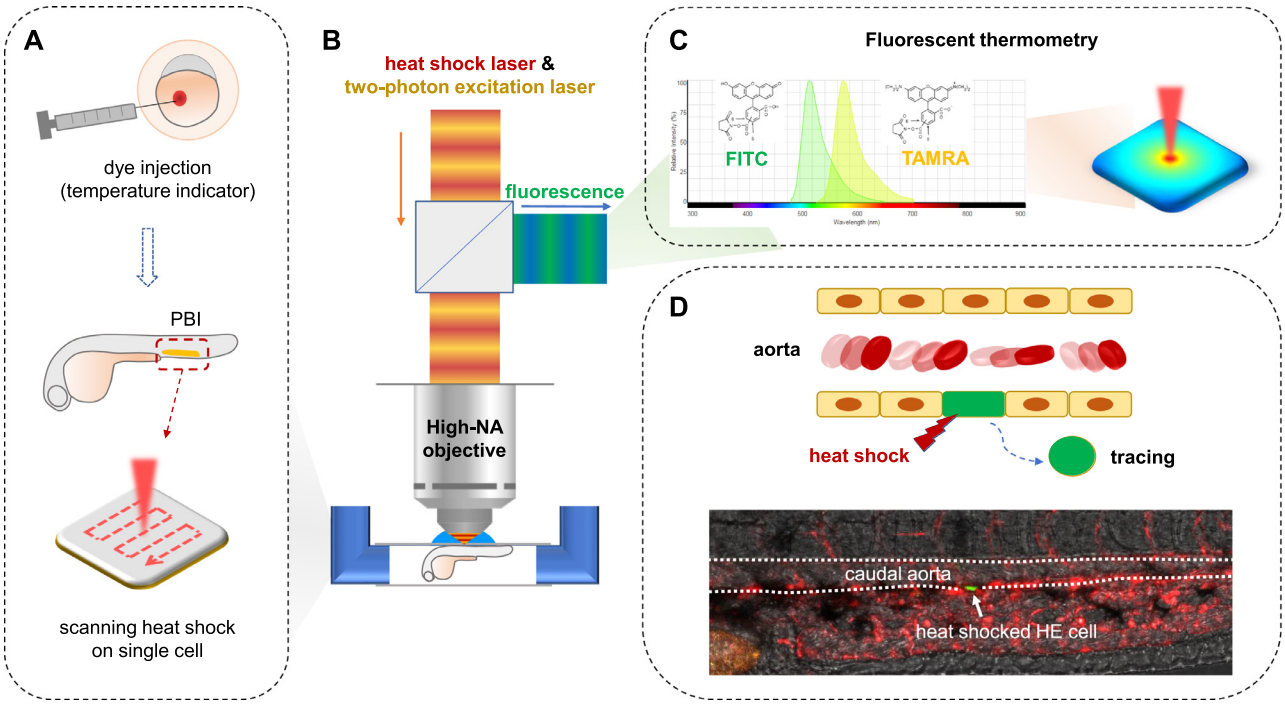

Figure 2. Schematics of the single-cell IR-LEGO system. **(A)** Fluorescent dyes (FITC and TAMRA) are injected into zebrafish embryos to serve as temperature indicators in tissues. Heat shock of a specific cell is generated by infrared (IR) laser scanning over the targeted cell to avoid cell damage. **(B)** The heat shock microscope system can tightly focus the IR laser on zebrafish tissues through an objective with a high numerical aperture (NA). A femtosecond laser is simultaneously employed to excite the fluorescent dyes (temperature indicator) in the targeted cell. **(C)** The two-photon fluorescence is subsequently analyzed by spectroscopy to calculate the cellular temperature (fluorescent thermometry). **(D)** Lineage tracing of a single hematopoietic endothelial (HE) cell. The heat shock is performed on a single HE at the aortic floor of the posterior blood island (PBI) of zebrafish embryos. The IR-irradiated embryo is then raised to a desired stage, and the contribution of the labeled HE to hematopoietic lineages is recorded.

(NA) was employed to tightly focus the infrared laser beam into the zebrafish so that a nearly point heat source would be generated inside the tissue. We then developed a cutting-edge two-photon fluorescence thermometry (TPFT) technique to noninvasively measure the three-dimensional cellular temperature raised by the infrared laser in a living zebrafish with high accuracy. In brief, thermally sensitive tetramethylrhodamine (TAMRA) and thermally insensitive fluorescein isothiocyanate (FITC) dyes were injected into zebrafish embryos. Because the two-photon excited fluorescence (TPEF) intensity ratio of TAMRA and FITC is linearly correlated with solution temperature, the peak temperature and volume of thermal diffusion in the heat-shocked cells could then be measured and serve as a gold standard to objectively optimize the single-cell labeling condition for a specific cell type. Under this optimized irradiation condition, the heat diffusion generated by the infrared laser in the zebrafish tissues would be restricted in a single-cell dimension. Finally, we adopted a scan heating mode by constantly scanning the infrared laser over the whole target cell for 32 sec (an optimized duration to label cells but avoid cell damage) to minimize the laser injury, thereby producing a fine balance between labeling efficiency and cell viability. With this strategy, we have been able to achieve single-cell labeling in different tissues of zebrafish, including a single myocyte in the skeletal muscle (80-mW infrared laser heat shock, 54.5% success rate), a single neuron in the central nervous system (95-mW infrared laser heat shock, 100% success rate), and a single leukocyte in hematopoietic tissues (80-mW infrared laser heat shock, 77.8% success rate). To have a proof of concept of this improved IR-LEGO system, we labeled and traced a single hematopoietic endothelial (HE) cell in the aortic floor of the posterior blood island in zebrafish, where hematopoietic progenitors are known to emerge [29,32]. The fate mapping result reveals that HE cells are heterogeneous and contain at least two subpopulations: one gives rise to myeloid progenies exclusively and the other produces both T lymphocytes and myeloid cells [31]. An intriguing issue for single-cell resolution fate mapping is the interference of background noise, which arises from occasional activation of the heat shock promoter in zebrafish without heat shock in this case. To overcome this problem, we employed a maximum likelihood estimation (MLE) method to depict the progeny of the targeted single cell with high fidelity. MLE is a statistical method used to extract desired information in the presence of background noise [33]. Indeed, the utilization of MLE significantly improves the fidelity of the single-cell IR-LEGO system [31]. On the other hand, considering that a single HSC is capable of giving rise to a large number of progenies, this background noise should have little influence, if any, on HSC lineage tracing. The current IR-LEGO system does have a few limitations. For example, it is more applicable to transparent samples, and the efficiency of single-cell labeling in some tissues is relatively low. Further refinement of the IR-LEGO system will be required to solve these problems. Nevertheless, we believe that this single-cell IR-LEGO system provides a powerful tool to study not only hematopoiesis but also stem cell biology in general.

In sum, the improved IR-LEGO system has indeed reached the highest resolution of cell labeling. With this unique system, information on the origins of hematopoietic progenitors and their differentiation potentials can be integrated, allowing us to address some long-lasting questions such as whether HSCs are generated in multiple positions and what kind of progenies can be generated from non-HSC progenitors from new dimensions.

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References

1. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell. 2008;132:631–644.
2. Bertrand JY, Jalil A, Klaine M, Jung S, Cumano A, Godin I. Three pathways to mature macrophages in the early mouse yolk sac. Blood. 2005;106:3004–3011.
3. Palis J, Robertson S, Kennedy M, Wall C, Keller G. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development. 1999;126:5073–5084.
4. Dharampuriya PR, Scapin G, Wong C, John Wagner K, Cillis JL, Shah DI. Tracking the origin, development, and differentiation of hematopoietic stem cells. Curr Opin Cell Biol. 2017;49:108–115.
5. Ghosn E, Yoshimoto M, Nakauchi H, Weissman IL, Herzenberg LA. Hematopoietic stem cell-independent hematopoiesis and the origins of innate-like B lymphocytes. Development. 2019;146.
6. Inman KE, Downs KM. The murine allantois: Emerging paradigms in development of the mammalian umbilical cord and its relation to the fetus. Genesis. 2007;45:237–258.
7. Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. Dev Cell. 2005;8:365–375.

8. Ottersbach K, Dzierzak E. The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. Dev Cell. 2005;8:377–387.
9. Samokhvalov IM, Samokhvalova NI, Nishikawa S. Cell tracing shows the contribution of the yolk sac to adult haematopoiesis. Nature. 2007;446:1056–1061.
10. Li Z, Lan Y, He W, et al. Mouse embryonic head as a site for hematopoietic stem cell development. Cell Stem Cell. 2012;11:663–675.
11. Sheng J, Ruedl C, Karjalainen K. Most tissue-resident macrophages except microglia are derived from fetal hematopoietic stem cells. Immunity. 2015;43:382–393.
12. Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature. 2015;518:547–551.
13. Naik SH, Schumacher TN, Perie L. Cellular barcoding: a technical appraisal. Exp Hematol. 2014;42:598–608.
14. McKenna A, Findlay GM, Gagnon JA, Horwitz MS, Schier AF, Shendure J. Whole-organism lineage tracing by combinatorial and cumulative genome editing. Science. 2016;353. aaf7907.
15. Pei W, Feyerabend TB, Rossler J, et al. Polylox barcoding reveals haematopoietic stem cell fates realized in vivo. Nature. 2017;548:456–460.
16. Henninger J, Santoso B, Hans S, et al. Clonal fate mapping quantifies the number of haematopoietic stem cells that arise during development. Nat Cell Biol. 2017;19:17–27.
17. Yu VWC, Yusuf RZ, Oki T, et al. Epigenetic memory underlies cell-autonomous heterogeneous behavior of hematopoietic stem cells. Cell. 2016;167:1310–1322. e1317.
18. Zhou F, Li X, Wang W, et al. Tracing haematopoietic stem cell formation at single-cell resolution. Nature. 2016;533:487–492.
19. Nestorowa S, Hamey FK, Pijuan Sala B, et al. A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. Blood. 2016;128:e20–e31.
20. Stringham EG, Candido EP. Targeted single-cell induction of gene products in *Caenorhabditis elegans*: a new tool for developmental studies. J Exp Zool. 1993;266:227–233.
21. Harris J, Honigberg L, Robinson N, Kenyon C. Neuronal cell migration in *C. elegans*: regulation of Hox gene expression and cell position. Development. 1996;122:3117–3131.
22. Halfon MS, Kose H, Chiba A, Keshishian H. Targeted gene expression without a tissue-specific promoter: creating mosaic embryos using laser-induced single-cell heat shock. Proc Natl Acad Sci USA. 1997;94:6255–6260.
23. Halloran MC, Sato-Maeda M, Warren JT, et al. Laser-induced gene expression in specific cells of transgenic zebrafish. Development. 2000;127:1953–1960.
24. Leitz G, Fallman E, Tuck S, Axner O. Stress response in *Caenorhabditis elegans* caused by optical tweezers: wavelength, power, and time dependence. Biophys J. 2002;82:2224–2231.
25. Ramos DM, Kamal F, Wimmer EA, Cartwright AN, Monteiro A. Temporal and spatial control of transgene expression using laser induction of the hsp70 promoter. BMC Dev Biol. 2006;6:55.
26. Xu J, Zhu L, He S, et al. Temporal–spatial resolution fate mapping reveals distinct origins for embryonic and adult microglia in zebrafish. Dev Cell. 2015;34:632–641.
27. He S, Chen J, Jiang Y, et al. Adult zebrafish Langerhans cells arise from hematopoietic stem/progenitor cells. Elife. 2018;7:e36131.
28. Kamei Y, Suzuki M, Watanabe K, et al. Infrared laser-mediated gene induction in targeted single cells in vivo. Nat Methods. 2009;6:79–81.
29. Tian Y, Xu J, Feng S, et al. The first wave of T lymphopoiesis in zebrafish arises from aorta endothelium independent of hematopoietic stem cells. J Exp Med. 2017;214:3347–3360.
30. Deguchi T, Itoh M, Urawa H, et al. Infrared laser-mediated local gene induction in medaka, zebrafish and *Arabidopsis thaliana*. Dev Growth Differ. 2009;51:769–775.
31. He S, Tian Y, Feng S, et al. In vivo single-cell lineage tracing in zebrafish using high-resolution infrared laser-mediated gene induction microscopy. Elife. 2020;9:e52024.
32. Bertrand JY, Kim AD, Violette EP, Stachura DL, Cisson JL, Traver D. Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. Development. 2007;134:4147–4156.
33. Shao J. Mathematical Statistics. Berlin/New York: Springer-Verlag; 2003.