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News & Views

Single-cell sequencing delivers hematopoietic stem cell specification

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Recently, Zhou et al. [1] have successfully traced the hematopoietic stem cell (HSC) specification during the stepwise development of HSC from the pre-HSC stage at single-cell resolution. These findings offer fundamental insights into mouse HSC fate decision and provide extensive information that may lead to novel discoveries in HSC differentiation.

HSCs, standing at the apex of the hierarchy organized by blood cells in high coordination, are capable of both giving rise to entire mature blood lineages and self-renewing ability. The identification of HSC was traced back to the studies on searching the cells capable of protecting humans exposed to minimum lethal doses of irradiation or chemotherapy initiated in 1940s [2]. Thomas et al. [3] performed bone marrow transplantations between identical twins and found the one with refractory leukemia who received the bone barrow from his twin brother exhibited hematologic recovery. Based on these findings, they proposed that there was a population of radioprotective cells that is capable of self-renew and multilineage differentiation existing in the bone marrows. A series of following studies confirmed the bone marrow contained highly proliferative progenitor cells that could give rise to myeloid, erythroid, megakaryocytic cells, as well as lymphocytes. Weissman lab firstly purified the blood forming stem cells in both mouse and human based on the expression of increasingly sophisticated pattes of cell surface markers [4, 5].

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Extensive efforts have been made to study the HSC biology and development since its isolation. Now overwhelming evidences have suggested hematopoiesis begins in the yolk sac blood islands, then in the dorsal aorta of the aorta-gonad-mesonephros (AGM) region and the placenta, eventually seeding into liver, spleen and then bone marrow. At least three HSC-competent cells have been identified as precursors of HSC in AGM region around E11, including haemogenic endothelial cells (ECs), $CD45^{-}$ pre-HSCs (T1 pre-HSCs), which prime CD41 expression and losing endothelial potential concurrently, and $CD45^{+}$ pre-HSCs (T2 pre-HSCs). T2 pre-HSCs proceed further and ultimately into mature HSCs [6, 7]. Although rapid advances in understanding the biological nature of hematopoietic stem cell have been achieved, the mechanisms that control lineage commitment and self-renewal of hematopoietic stem cells are still ambiguous. The bottlenecks restricted the development in this field mainly raise from the rarity (fewer than 1 hematopoietic stem cell in 10,000 fetal liver and adult bone marrow cells) and heterogeneity of the HSC and pre-HSC populations. The pre-HSCs vary fast and continuously in early development and thus it's hard to exactly capture the pre-HSCs in mouse mid-gestation embryos. Furthermore, the complexity of surface markers of HSC and pre-HSC populations challenges the function evaluation using engraftment and lineages chimerism after transplantation. Zhou et al. [1] paved a way for dissection of complex molecular mechanisms regulating stepwise generation of HSCs in vivo by combining a new combination of surface markers to isolate the T1 and T2 pre-HSC in AGM and single cell RNA sequencing.

Cellular heterogeneity is a challenge to understand stem cells. Traditional high-throughput sequencing provides an average view of the transcriptome across many cells, and so cannot provide information about the characteristics of

rare cell types within a heterogeneous population. Thanks to rapid advancements in single-cell sequencing technologies, now this problem could be addressed in systematic and non-biased way [8]. Single cell sequencing can examine the transcriptome information from individual cells with optimized next generation sequencing technologies, providing a unprecedented opportunity for dissection of gene expression networks in rare cell types within a heterogeneous population [9–11].

To enrich T1 pre-HSC population, Zhou et al. first evaluated the efficacy of several candidate surface markers including VE-cadherin, AA4.1, and CD201. By combing the robust in vitro single-cell culture system and function evaluation assays, the $CD31^+$ $CD45^-$ $CD41^{low}$c-Kit^+ $CD201^{high}$ population in the E11 AGM region was identified to be T1 pre-HSC population that can be enriched efficiently. Then by using single-cell sequencing, the traditional T2 pre-HSC population isolated by $CD31^+$ $CD45^+$ $CD41^{low}$ from the E11 AGM region was identified to be contaminated with myeloid cell, and the $CD31^+$ $CD45^+$ c-Kit^+ $CD201^{high}$ population was corrected to be the genuine T2 pre-HSC which can directly give rise to HSCs.

Based on the accurate purification of the nascent pre-HSCs and HSCs with the potent surface markers, Zhou et al. executed single-cell RNA sequencing on five stages of HSC development (EC, T1 pre-HSC, and T2 pre-HSC in the E11 AGM region, E12 HSC and E14 HSC in the fetal liver). The authors then performed extensive analysis on dynamics in transcription networks, surface signature genes, metabolism states, cell circle features and signaling pathways during HSC formation using the single cell RNA-Seq data. A continuous developmental process from ECs to HSCs through T1 and T2 pre-HSCs was discovered with each stage a unique pattern of surface marker gene and transcription factor expression (Fig. 1). These results provided extensive information for uncovering mechanisms of HSC ontogeny from different angles. For example, the transcriptome analysis demonstrated a sharp increase expression of ribosome-based translational machinery from ECs to T1 pre-HSCs and a gradual transcriptional decrease from pre-HSCs to HSC. Gene ontology (GO) analysis

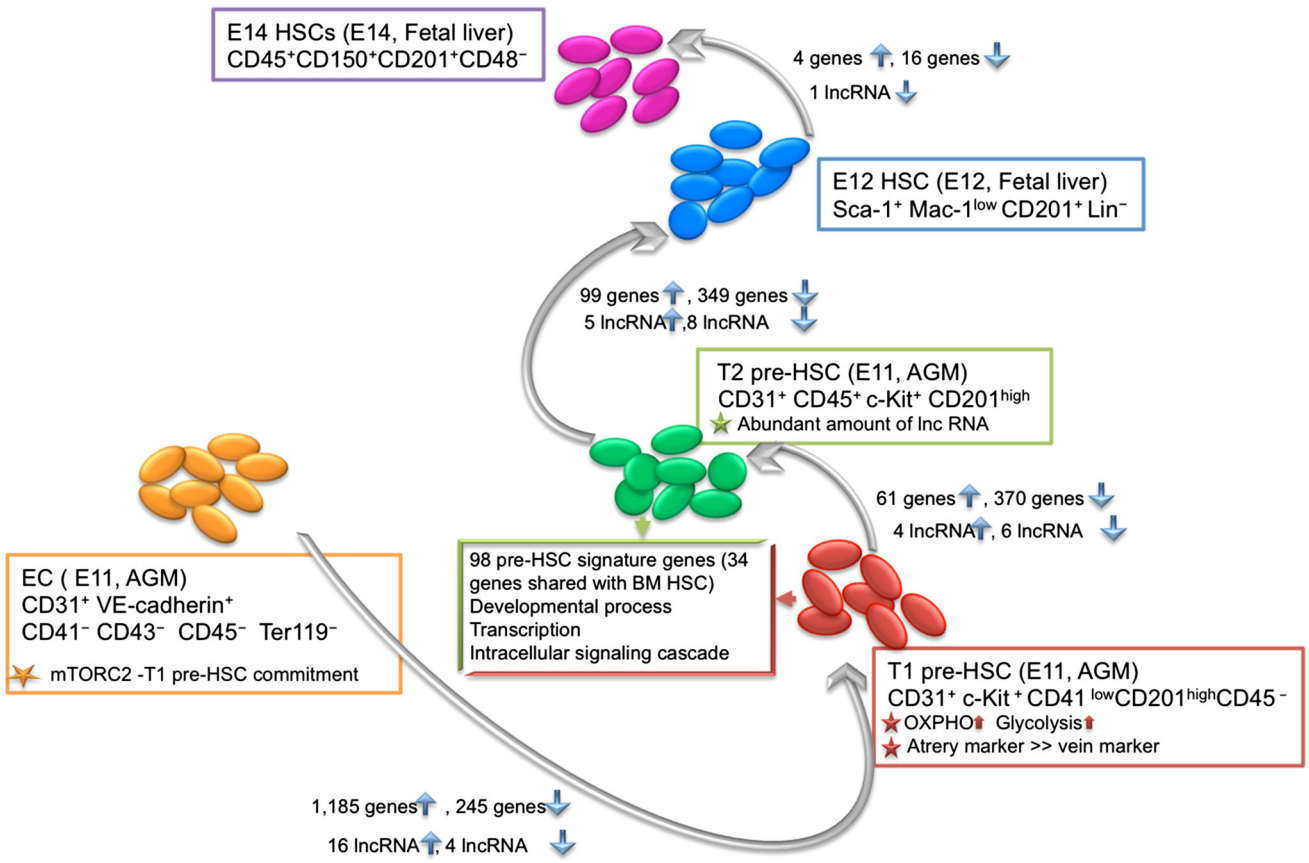

Fig. 1 (Color online) The stepwise road for HSC specification. Single-cell RNA sequencing reveals each developmental stage during HSC formation (EC, T1 pre-HSC, and T2 pre-HSC in the E11 AGM region, E12 HSC and E14 HSC in the fetal liver) has a unique pattern of gene expression respectively, indicating each developmental process needs a comprehensively cooperated regulation in transcription networks, surface signature genes, metabolism states, cell circle features and signaling pathways etc

revealed that the most dramatic changes occurred between ECs and T1 pre-HSCs, characterized by down-regulation of the genes related to cell migration, vasculature development, and blood vessel morphogenesis, and up-regulation of genes related to hematopoietic or lymphoid organ development and intracellular signaling cascade (Fig. 1). The arterial signature of T1 pre-HSCs suggests that pre-HSCs should have a more intimate lineage relationship with arterial ECs than with venous ECs. Interestingly, mTOR signaling pathways identified by single cell sequencing was functionally verified to be required for HSC specification, indicating the feasible strategy to dig novel mechanisms using single-cell sequencing data.

This is the first integrated assay that the transcriptome of pre-HSCs and HSCs during embryonic development have been comprehensively surveyed at single-cell and single-base resolution. This study provides a paradigm to delineate cell fate decision mechanisms on cells with elusive heterogeneity. Stem cells hold great promise for therapy [12, 13]. The HSCs have been successfully applied in treatment of hematopoietic diseases such as leukemia, anaemia and congenital immunodeficiency et al. However, the application of HSCs is restricted by the limitary resource. In vitro induction of human ESCs/iPSCs into functional HSCs remains a challenge in this field, partially due to the insufficient understanding on human HSC ontogeny [14, 15]. Two recent researches have generated the global gene regulation dynamics data during both in vitro process of hematopoietic differentiation and reprogramming by high-throughput sequencing at cell population level, respectively [16, 17], providing additional data resource for dissection hematopoiesis. Given the successful repopulation of human immune system in NSG mice by transplantation of human $CD34^+$ cells and thymus [18], the single-cell sequencing technology offers great opportunity to delineate the ontogeny of human HSCs, which will significantly benefit clinical development of human HSCs.

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Conflict of interest The authors declare that they have no conflict of interest.

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