RESEARCH HIGHLIGHT

Hematopoietic heterogeneity starts at the hemogenic endothelium

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Hematopoietic stem cells are heterogenous during development and adulthood; however, when and how this heterogeneity begins is still not well understood. In a new study published in *Cell Research*, Xia et al. show that hemogenic endothelial cells are heterogeneous and the transcription factor SPI1 in mammals or Spi2 in zebrafish is partially responsible for the establishment of this heterogeneity.

Hematopoiesis is the process of blood generation that starts during development in several waves. The primitive wave gives rise to unipotent blood cells, mostly erythroid and myeloid cells. The intermediate wave gives rise to multipotent hematopoietic progenitors. The definitive wave generates hematopoietic stem and progenitor cells (HSPCs) on the ventral part of the dorsal aorta. In vertebrates, definitive hematopoiesis occurs through an endothelial-to-hematopoietic transition (EHT) where an endothelial cell (EC) becomes hemogenic endothelial cell (HEC) and finally HSPC. HSPCs later translocate to the caudal hematopoietic tissue (CHT) in zebrafish or the fetal liver in mammals and later seed the adult hematopoietic organs.[1]

A growing amount of evidence in the last years has shown that HSPCs are heterogeneous. The use of different methods such as cell tracking after labeling,[2] single-cell transcriptomics (scRNA-seq),[2,3] or in situ barcoding techniques[2,4] allowed to highlight the heterogeneity of HSPCs in vivo. Given that embryonic HSPCs derive from HECs, this heterogeneity could be pre-determined at the HEC stage. In that sense, by using fate mapping, HECs in zebrafish embryos have been shown to possess different hematopoietic lineage outcomes.[5] Still in zebrafish, clonal analysis through fate mapping assays using the zebrabow labeling system demonstrated lineage heterogeneity of embryonic hematopoietic stem cells (HSCs) with up to 30 HSC clones present at peak production from the aortic endothelium.[2] In vivo studies in mouse embryos have revealed that embryonic-born HSCs and progenitors are generated from distinct HECs[3] and have distinct differentiation potential and lifelong contribution.[4] For example, genetic regulation of microRNA-223-mediated *N*-glycan biosynthesis modulates the heterogeneous fate determination of HSPCs during EHT.[6] Furthermore, in 2022, by using in situ barcoding in addition to classical fate mapping, Patel and colleagues identified that embryonic-born multipotent progenitors can contribute to young adult hematopoiesis in mice, independent of traditional HSCs.[4] HSC heterogeneity could therefore be the result of HEC heterogeneity, although it appears that the mechanism remains unclear.

In a new study in *Cell Research,*[7] scRNA-seq and chromatin accessibility assays (scATAC-seq) in endothelial and hematopoietic compartments of zebrafish embryos led to the identification of lymphoid-, myeloid- and erythroid-biased HSPCs (L-, M-, E-HSPCs). Moreover, further scrutiny of the data revealed the existence of heterogenous HECs and most importantly pre-HEC, lymphoid/myeloid-biased HEC (L/M-HEC), and erythroid-biased HEC (E-HEC). Indeed, trajectory analysis using RNA velocity showed a continuous path from arterial EC through heterogenous HECs to heterogenous HSPCs. The authors then tried to identify transcription factors responsible for the HEC heterogeneity. One of the most prominent motifs that was identified in highly accessible genomic regions in HECs and L/M-HSPCs was the SPI1 motif. Indeed, the homolog of SPI1 in zebrafish, Spi2, was the most highly expressed member of the SPI1 family (Fig. 1).

Transcription factors are pivotal for HSC differentiation and maintenance. One of the most important factors not only for terminal myeloid differentiation, B and T cell development, but also for HSC maintenance and stemness is SPI1. Deletion of *SPI1* during development showed that HSCs lacking SPI1 could not compete with their wild-type counterparts and could contribute only transiently to the HSC pool upon transplantation to adult recipients.[8] In the present work, lineage tracing of $Spi2^+$ cells showed that they can be readily detected in HECs and HSPCs, and time-lapse imaging showed their expression in the CHT and the thymus. Elimination of the $Spi2^+$ cells led to a complete absence of positive cells in CHT and a great reduction in the thymus as well as a decrease in the numbers of L/M-HSPCs but not that of E-HSPCs. Indeed, significant enrichment for L/M signatures was found in $Spi2^+$ cells. This is in agreement with previous publications showing that SPI1 levels also highlight the HSC heterogeneity with SPI1-low HSCs exhibiting long-term repopulating activity and SPI1-high HSCs exhibiting myeloid lineage priming.[9] Thus, even though work on the role of SPI1 in HSC heterogeneity exist, this was the first evidence that SPI1 may play a role in HEC heterogeneity.

Recently, it was shown that SPI1 enforces quiescence and limits HSPC expansion upon inflammatory stress.[10] Here in a model of bacterial infection by injection of LPS, elevated numbers of $Spi2^+$ cells with increased markers for HSPCs and L/M traits were observed. Deletion of *spi2* led to diminished numbers of HECs and HSPCs in the dorsal aorta which could be rescued by EC-specific overexpression of *spi2*, thus providing evidence that Spi2 regulates HEC/HSPC generation. scRNA-seq of ECs, HECs, and HSPCs showed

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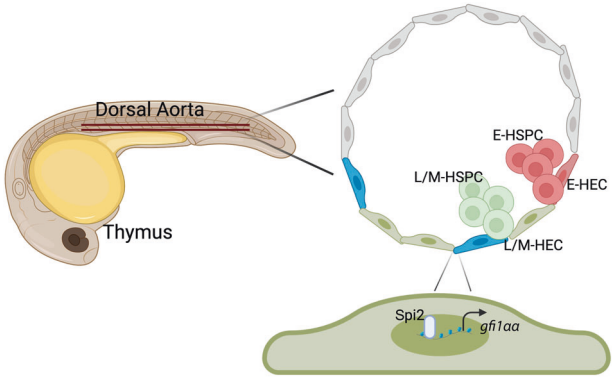

Fig. 1 Hematopoietic heterogeneity begins at the level of HECs. Indeed, different types of E-HEC and L/M-HEC exist and give rise to E-, L-, and M-HSPCs. Heterogeneity at the level of HECs and HSPCs is controlled by Spi2, a zebrafish homolog of the mammalian SPI1 transcription factor, at least partly through the regulation of *gfi1aa*, a homolog of the mammalian *GFI1*.

that the HEC/HSPC cluster was almost absent in *spi2* morphants with the most prominent decrease in L/M-HECs and L/M-HSPCs but not in E-HECs or E-HPSCs.

The direct modulation of HEC/HSPC fate by Spi2 was proven by CUT&TAG experiments where the binding motif of Spi2 was identified as the most enriched. Significant overlap was found between the bound and deregulated genes. One of these bound and deregulated genes was *gfi1aa*, the zebrafish homolog of *Gfi1* that could rescue the HSPC defects in *spi2*$^{-/-}$ embryos, proving that it is a direct target of Spi2 and important for controlling HSPC fate choice in HECs.

Finally, to prove the relevance to the human system, the authors showed that SPI1 was highly expressed in the human dorsal aorta; while the transcriptomic signature of SPI1-positive HECs was associated with hematopoietic cell function, the SPI1-negative HEC signature was enriched for endothelium development. Similarly, in a system of human induced pluripotent stem cell differentiation, it was found that HECs and HSPCs expressed higher levels of SPI1 compared to ECs, and that SPI1 knockdown led to reduced formation of granulocyte-macrophage but not erythrocyte colonies.

It would be interesting in the future to look for further heterogeneity upstream of the hemogenic endothelium in the ECs and examine the role of potential transcription factors that regulate heterogeneity. As SPI1 is a pioneering transcription factor for myeloid lineages, it would be interesting to know whether other factors like the erythroid transcription factor GATA1 is, for example, responsible for the generation of E-HECs.

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