Opening the window for endothelial-to-hematopoietic transition

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Definitive long-term hematopoietic stem cells (LT-HSCs) arise during embryogenesis in a process termed endothelial-to-hematopoietic transition (EHT), in which specialized hemogenic endothelial cells (HECs) transform into hematopoietic cells. The transcription factor RUNX1 marks HECs and is essential for EHT. Ectopic *RUNX1* expression in non-HECs is sufficient to convert them into HECs. However, the conversion efficiency depends on the developmental timing of expression. In this issue of *Genes & Development*, Howell and colleagues (pp. 1475–1489) leverage this observation to further understand how RUNX1 mediates EHT. They engineered mice that ectopically express *RUNX1* in endothelial cells at different developmental time points and doses. They then performed chromatin accessibility and other analyses and correlate this with hemogenic potential. They found that RUNX1 collaborates with TGFβ signaling transcription factors to drive chromatin accessibility changes that specify HECs. They also highlight interesting parallels between EHT and endothelial-to-mesenchymal transition (EndoMT), which occurs during cardiac development. The results of Howell and colleagues provide new mechanistic insights into EHT and take us one step closer to generating patient-specific LT-HSCs from induced pluripotent stem cells.

Definitive long-term hematopoietic stem cells (LT-HSCs) provide life-long blood cell production for an organism. They arise during a brief developmental window in embryogenesis, which in mice occurs around embryonic days E9.5–E10.5. During this process, specialized "hemogenic" endothelial cells (HECs) located within the ventral aspect of the dorsal aorta, as well as several other vessels, undergo a process termed endothelial-to-hematopoietic transition (EHT) (Fig. 1; Kissa and Herbomel 2010). This involves loss of endothelial and gain of hematopoietic gene programs. During this transition, HECs lose their tight junctions, round up, and bud off into circulation as hematopoietic stem and progenitor cells (HSPCs). These then seed the fetal liver and later the bone marrow to sustain hematopoiesis.

The runt family transcription factor RUNX1 is essential for EHT, and its expression distinguishes HECs from non-HECs (North et al. 1999). Moreover, ectopic *RUNX1* expression in non-HECs is sufficient to convert them into HECs (Eliades et al. 2016; Yzaguirre et al. 2018). However, the conversion efficiency is highly dependent on the developmental timing of the ectopic expression. Ectopic expression at E7.5–E8.5 leads to efficient conversion, whereas expression at later stages (E9.5 and later) produces much lower conversion, if at all. This indicates that additional factors modulate the competency for RUNX1 to initiate EHT.

In this issue of *Genes & Development*, Howell et al. (2021) leverage the observations above to further understand the mechanisms by which RUNX1 mediates EHT. The investigators engineered mice to ectopically express *RUNX1* in endothelial cells (ECs) at different developmental time points. This involved knock-in of the *RUNX1* cDNA, preceded by a loxP-flanked translational stopper cassette, into the *Rosa26* locus. Incorporation of a tamoxifen-inducible vascular endothelial cadherin-Cre transgene allowed them to conditionally express *RUNX1* in ECs. By using mice containing one or two *RUNX1* knock-in alleles, they could examine the impact of RUNX1 dosage. The investigators induced *RUNX1* expression at E8.5 or E12.5. Embryos were harvested 24 h later, and their ECs were assayed for hemogenic potential and further characterized.

When using a single *RUNX1* allele, induction at E8.5 gave rise to HECs with a frequency of one in 58 ECs, whereas induction at E12.5 gave essentially no conversion, consistent with prior work. Use of a higher RUNX1 dosage (two alleles) at E12.5 converted some ECs, although the efficiency was lower (one in 185 cells) compared with a single *RUNX1* allele at E8.5. The investigators then began
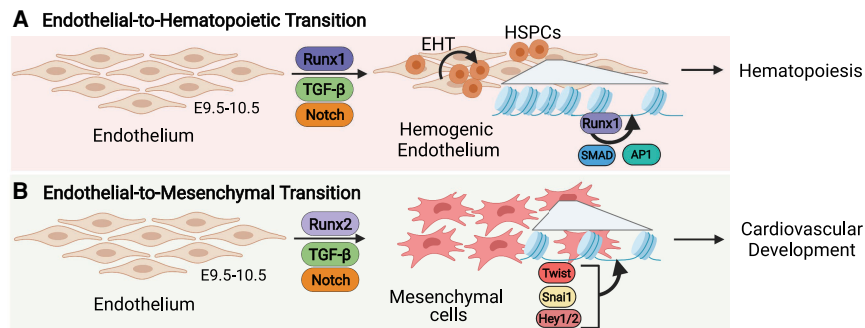
Figure 1. Parallels between endothelial-to-hematopoietic transition (EHT) and endothelial-to-mesenchymal transition (EndoMT). EHT and EndoMT occur at similar times during embryonic development. Both are driven by RUNX family transcription factors and involve TGF-β and NOTCH signaling. Howell et al. (2021) now show that RUNX1 collaborates with TGFβ signaling transcription factors AP-1 and SMAD2/3 to open chromatin at sites involved in EHT. Genes selectively accessible during EHT include *Twist*, *Snai1*, and *Hey1/2*, which play functional roles in EndoMT. (Figure created in BioRender).

probing mechanisms underlying the differential competency. Gene expression changes 24 h after induction were minimal, leading them to examine chromatin accessibility. Using ATAC-seq, they observed significant changes in accessibility, particularly at genes involved in stem cell and heart development, as well as endocardial cushion development/morphogenesis. These included genes enriched for TGFβ signaling targets. In vivo footprinting showed that at higher doses RUNX1 was able to access high-affinity binding sites that were normally located in closed chromatin at E13.5. Moreover, they demonstrated colocalization of RUNX1 and the TGF-β signaling transcription factors SMAD2/3 and AP-1 at these sites. These findings suggested cooperativity between RUNX1 and TGF-β signaling transcription factors. To test this, they exposed E13.5 derived ECs to TGFβ3 and showed that this enhanced the efficiency of RUNX1-mediated conversion to HECs.

Taken together, the study provides a number of significant findings. First, it suggests that RUNX1 facilitates chromatin opening to allow recruitment of additional factors that drive hemogenic conversion. This activity is RUNX1 dosage-dependent, implying that regulation of RUNX1 levels likely plays an important role in EHT spatiotemporal control. It is also another example of a RUNX family member acting as a pioneer transcription factor (Lee et al. 2019).

Second, it demonstrates critical interplay between RUNX1 and TGF-β signaling to facilitate chromatin reorganization, particularly at genes involved in the morphogenic aspects of EHT. This work complements an earlier study in zebrafish showing a requirement for TGFβ signaling in EHT (Monteiro et al. 2016). Reciprocal cooperation between RUNX1 and AP-1 has also been described in megakaryopoiesis (Pencovich et al. 2011). It will be of interest to further characterize the sources of TGFβ signaling in mammalian systems.

Third, the investigators highlight interesting parallels between EHT and the endothelial-to-mesenchymal transition (EndoMT), which is involved in cardiac development (see Fig. 1; Kovacic et al. 2019). Both processes occur around the same developmental window and result in decommissioning of endothelial gene programs. Both lead to weakened tight junctions and increased migratory behavior. RUNX1 is required for EHT, whereas the family member RUNX2 drives EndoMT (Tavares et al. 2018). Last, both processes use TGF-β and NOTCH signaling.

One of the caveats of the work by Howell et al. (2021) is that several other studies have demonstrated inhibitory effects of TGF-β signaling on EHT. The investigators attribute this discrepancy to the highly sensitive nature of the TGF-β response that is largely dependent on the concentration and duration of treatment. There may also be concerns about an inhibitor used in some of the earlier studies. It is also not clear yet that the cells produced by ectopic RUNX1 expression and TGFβ stimulation include functional HSCs. These points will need to be clarified in future work.

A long-term goal in the regenerative medicine field is to generate LT-HSCs from human induced pluripotent stem cells in vitro. However, reliable and efficient methods remain a challenge. Improved mechanistic understanding of how LT-HSCs normally arise during development should greatly facilitate these efforts. Thus, the work of Howell et al. (2021) takes the field one step closer to that goal.

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