this population in mouse embryonic stem cell differentiation cultures depends on activation of both Hedgehog and Notch signaling during the stages of mesoderm formation and specification. They also provided evidence that Hedgehog signaling acts upstream of the key hematopoietic transcriptional regulator Scl, which is required for the EHT. This work underscores the complexity of embryonic hematopoietic development and highlights the distinct temporal requirements of the various signaling pathways to ensure proper specification of mesoderm to hemogenic endothelium and subsequently to hematopoietic progeny.

These recent studies[1–4] contain many new insights into the complexities of hematopoietic development. Yet a major challenge in modeling hematopoiesis with PSCs is the fact that the primitive hematopoietic program often emerges first and dominates the cultures, masking and possibly inhibiting the development of definitive hematopoiesis. Complicating the issue further is the observation from studies with mouse PSCs that the hemangioblast seems to transition through a hemogenic-endothelium intermediate stage as it differentiates to its primitive hematopoietic progeny[11]. These findings raise the cautionary note that the appearance of hemogenic endothelium in PSC differentiation cultures does not necessarily indicate the onset of definitive hematopoiesis and emphasize the need to incorporate assays into the experiments that can distinguish the two hematopoietic programs.

Using T-lymphoid potential to monitor definitive hematopoietic development in hPSC cultures, we recently demonstrated that primitive hematopoiesis and definitive hematopoiesis differ in their requirement for activin/nodal signaling at early stages of differentiation: the primitive program depends on this pathway, whereas the definitive program does not[5] (**Fig. 1**). We found that definitive hematopoiesis arose from a $CD34^+$ population that displayed the same hemogenic cell-surface phenotype as identified by Choi *et al.*[1]. The differences in activin/nodal signaling requirements between the two hematopoietic programs provide a simple approach for producing populations enriched in definitive hematopoietic progenitors, enabling detailed analyses of hemogenic endothelium development and EHT in the absence of contaminating primitive hematopoietic cells.

The ability to generate populations enriched in definitive hematopoietic progenitors provides an opportunity to identify cell-surface markers that distinguish progenitors of the two hematopoietic programs. These markers can then be added to the consensus set of surface markers used to define hemogenic endothelium and its hematopoietic progeny, enabling one to easily distinguish primitive and definitive populations within differentiation cultures. Use of such protocols and reagents will ensure that different groups are studying similar populations.

With populations enriched in definitive hematopoietic progenitors and appropriate marker combinations, one will be able to separate hemogenic endothelium in these cultures from nonhemogenic endothelium. The ability to isolate the two types of endothelium would represent an important step forward as it would enable one to study the signaling pathways that regulate their development and to determine whether hemogenic endothelium is specified through a nonhemogenic endothelial cell intermediate or directly from mesoderm.

With access to enriched populations of definitive hemogenic endothelium, it will be possible to investigate in detail the signaling pathways that regulate the EHT, the development of different hematopoietic populations and, ultimately, the generation of HSCs. Such approaches will have to test both activation and inhibition of a range of different pathways. BMP, WNT and SHH are widely used in the induction of hematopoietic mesoderm but are not often applied in later-stage differentiation cultures, even though they are known to be required for definitive hemogenic endothelium specification and HSC emergence. Similarly, NOTCH likely plays a biphasic role, being essential not only for the induction of hemogenic endothelium specification but also for the EHT[12].

In all cases, signaling pathways will have to be modulated in a time- and concentration-dependent manner to recapitulate embryonic development. Additionally, the contributions of other differentiation cues, such as blood flow and shear stress[13] or catecholamines from the sympathetic nervous system[14], will have to be investigated systematically. Different culture formats (transwell, co-culture, two-dimensional, three-dimensional) can be used for different stages of hematopoietic development and interrogated to manipulate additional contact-mediated signaling requirements, such as NOTCH and integrin-mediated interactions. These approaches, combined with the use of targeted reporter PSC lines, will lead eventually to a highly detailed road map for the human definitive hematopoietic lineage, transitioning from mesoderm to hemogenic endothelium and, finally, to HSCs.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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