EPCR: a novel marker of cultured cord blood HSCs

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In this issue of Blood, Fares et al demonstrate that endothelial protein C receptor (EPCR) is a reliable marker of human cord blood (CB) hematopoietic stem cells (HSCs), both from uncultured cells and those expanded with UM171, a pyrimidoindole derivative previously shown to expand CB HSCs. These studies build on 2 prior observations, that exposure of CB-derived CD34+ cells to UM171 leads to a rapid induction of EPCR expression both at the messenger RNA and protein levels and that EPCR expression can be used to identify murine HSCs, the latter finding suggesting that EPCR may be used similarly in the human setting. Identifying methods to quickly assess expansion of human HSCs ex vivo is an important issue, as many groups have developed methods to expand CB hematopoietic stem and progenitor cells (HSPCs) ex vivo (reviewed in Mehta et al); however, as cultured HSCs frequently exhibit altered surface phenotypes, surrogate markers that reliably define HSCs expanded ex vivo need to be identified because assessment of long-term (LT) HSC activity from expanded cultures currently relies on time-consuming serial transplantation experiments in immunocompromised mice. Identifying markers that can identify HSCs rapidly would facilitate the development of high-throughput screening strategies to assess novel HSC ex vivo expansion methods as well as the identification of molecular pathways involved in HSC function.

In the present study, the authors demonstrate that only EPCR+ CD34+ CB cells express 2 previously identified human HSC markers, CD90 and CD133, indicating that EPCR expression defines a subset of HSCs with a more primitive phenotype (CD34+CD38−CD49medCD90−CD133−). Given the ability of EPCR to enrich for LT-HSCs in unmanipulated CB cells, the authors also tested whether EPCR identifies LT-HSCs in expanded CB cultures. The enrichment in repopulating activity among EPCR+ cells increased during culture from day 7 (20-fold relative enrichment, EPCR+ vs bulk) to day 12 (56-fold). Indeed, using limiting dilution analyses, the authors showed that UM171-expanded cultures from day 7 cultures contained EPCR+ cells enriched for functional multipotent LT-HSCs (1 in 68 cells) compared with EPCRlow+ cells (1 in 2016) or EPCR− cells (1 in 4240). In addition, the authors confirmed that CB-enriched HSPCs are hierarchically organized, with EPCR+ CD34+ cells giving rise to EPCR− CD34+ cells, but not vice versa. EPCR+ CD34+ cells also exhibited self-renewal, as only EPCR+ cells were able to engraft secondary recipients. Together, these studies showed that EPCR marks functional HSCs.

To determine if EPCR is essential for HSCP function, EPCR was silenced with targeted short hairpin RNA vectors or ectopically expressed. Although EPCR has been shown to regulate mouse LT-HSC bone marrow (BM) and mice expressing low levels of EPCR (Protein) showed defects in HSC BM homing associated with increased levels of circulating HSCs, the authors showed that loss or overexpression of EPCR had no impact on the expression of other HSC markers, cell proliferation, or the ability to repopulate NOD-Scid IL-2Rγ null (NSG) mice, suggesting that EPCR may not be required for HSCP function in humans (see figure). To determine the molecular differences among ex vivo expanded CB cells, the authors compared the transcriptomes of EPCR+ and EPCR− cell fractions sorted from UM171-treated CD34+CD45RA− CB cells. EPCR+ cells were enriched for HSC-associated genes compared with EPCRlow− cells, and the authors defined a 120-gene signature associated with EPCR expression composed of transcriptional regulators such as HLF, PRDM16, and MECOM, as well as genes encoding antigens previously described as expressed by HSCs including CD90, CD133, and GPR56. Collectively, these data demonstrate that EPCR is a novel marker that allows the identification and prospective separation of human LT-HSCs, both in unmanipulated and ex vivo UM171 expanded CB HSCs.

Although these studies represent an exciting advance in HSC biology, it will be important for others to confirm these results and test whether EPCR also can be used to prospectively separate HSC-enriched populations from CD34+ cells from adult BM and granulocyte-colony stimulating factor mobilized peripheral blood, particularly because it was previously reported that EPCR may not mark human BM-derived HSCs. In
Finally, as it is unclear which methods function such as time to engraftment and peripheral blood grafts with measures of HSPC frequency and function in xenograft models may not accurately reflect human CB HSCs. Such investigations have the potential to further credential EPCR as a general marker of ex vivo expanded CB HSCs. 

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.  

**REFERENCES**  

**LYMPHOID NEOPLASIA**

**Activated kinases in ALL: time to act**

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In this issue of *Blood*, Reshmi et al reported a study that defines a protocol for identifying kinase-driven high-risk (HR) features, known as “Ph-like” expression profile, in patients with acute lymphocytic leukemia (ALL). Revealing the underlying genetic aberration allows better prognostication and may point to potential therapeutic options for specific patients. Originally identified in pediatric patients, this Ph-like or kinase-driven ALL (KD-ALL) subtype has also been found to be common among adults. The journey to the routine identification of these kinase-activating genetic alternations started 8 years ago and required extensive efforts and use of different laboratory methods to become feasible. The most important take-home message from this work is that the time has come for routine screening for kinase-activating alterations in ALL. Although this study is published before clinical outcome data of the patients enrolled in the Children’s Oncology Group study have matured, the clinical significance of identification of KD-ALL is well established. Reshmi et al confirm the complexity of the genetic alteration map of these potentially targetable aberrations. The authors also provide a working diagnostic paradigm starting with a simple gene expression screening test, which reliably identifies patients in whom genetic testing for kinase-activating alterations is futile. Of 202 patients whose suggested score for screening was below 0.5, only in 1 was a potentially targetable fusion detected (HOOK3–FGFR1 genes).

Screening aims at identifying HR patients and patient-specific potentially druggable targets. The proposed laboratory protocol is complex. It requires the use of multiple sophisticated methods, is costly, and time consuming. Moreover, the expected...
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