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LETTER TO THE EDITOR

The niche for hematopoietic stem cell expansion: a collaboration network

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Transplantation of hematopoietic stem/progenitor cells (HSPC) has become one of the most effective treatments for several hematological malignancies, cancers and genetic immune disorders. However, the lack of histocompatible donor sources and the number of stem cells suitable for engraftment are major constraints in the clinic, and these constraints greatly hamper the use of stem cell therapy. In humans, fetal liver and bone marrow are the two major locations of hematopoiesis before and after birth, respectively. Thus, understanding the specialized stem cell microenvironment supporting the self-renewal and expansion of HSPC in fetal liver and bone marrow is not only essential for extending the current knowledge of stem cell biology but is also beneficial to adult regenerative medicine in the clinic.

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Despite intensive research over the past several decades, there has not been a clear overall picture of how the hematopoietic stem cell niche functions in the expansion of primitive and self-renewing HSPCs. Many studies have shown that the expansion of HSPCs depends on some well-known growth factors, such as stem cell factors, flt3 ligands, thrombopoietin, interleukin 6, interleukin-3 and angiopoietin-like 5, which function in different combinations,[1–3] and newly recognized factors such as notch ligand and stemregenin 1.[4,5] However, whether cytokines alone lead to HSPC expansion with self-renewing capacity remains unclear, as previous studies have also indicated that direct interactions between HSPCs and cellular components, such as blood vessel endothelial cells, osteoblasts, mesenchymal stem cells and so on, in the stem cell niche are crucial for the regulation of hematopoiesis.[6,7] Moreover, there are ongoing debates about whether HSPCs lose long-term grafting ability during *in vitro* expansion as a result of the loss of a proper stem cell-forming niche, which has not yet been accurately formatted in existing stem cell protocols.[8] New insights into the hematopoietic stem cell niche are required.

In a previous study, we showed that human hematopoietic stem cells in the fetal liver, cord blood and bone marrow, which carry the actual long-term *in vivo* repopulating activity, possess a unique phenotype of $CD34^{hi}CD133^{hi}$, while the $CD34^{hi}CD133^{neg}$ phenotype defines hematopoietic progenitor cells and only contributes to the differentiation of specific lineages.[9] According to another study, mesenchymal stem cells engineered to express growth factors can robustly support the expansion of $CD34^{hi}CD133^{hi}$ cells, and both cell-cell contact and soluble factors are essential for this process.[10] Yong *et al.*[11] recently identified a novel human liver progenitor cell population in the fetal liver with a unique phenotype of $CD34^{lo}CD133^{lo}$. This pluripotent cell population could give rise to multiple cell lineages, including hepatocytes, endothelial cells, adipocytes and osteocytes; thus, $CD34^{lo}CD133^{lo}$ cells contribute an essential component in the fetal liver niche. Furthermore, $CD34^{lo}CD133^{lo}$ cells express principal growth factors that are important for stem cell expansion such as stem cell factor, insulin-like growth factor 2, C–X–C motif chemokine 12 (CXCL12) and factors in the angiopoietin-like protein family. The co-culture of fetal liver $CD34^{lo}CD133^{lo}$ cells with autologous fetal liver $CD34^{hi}$ HSPCs or allogenic cord blood HSPCs supports the *ex vivo* expansion of HSPCs from both resources. In addition, this new co-culture-based expansion only relies on the soluble factors secreted from $CD34^{lo}CD133^{lo}$ cells and is cell-cell contact independent.

Considering these results, it is thought that there is a network of various cell

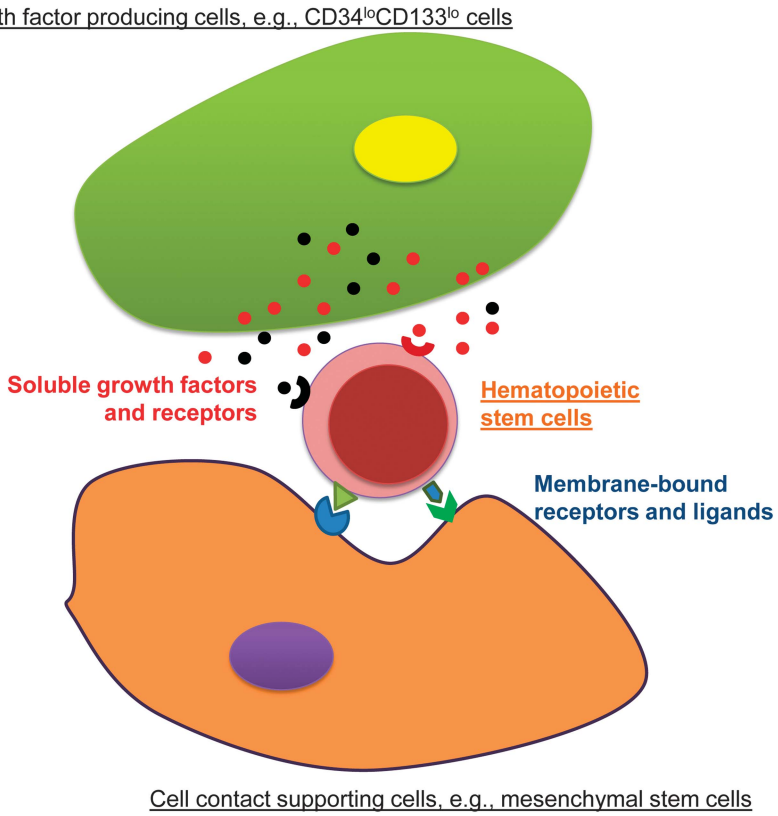

Figure 1 Two major types of feeder cells for HSPCs in a fetal liver stem cell niche.

types that reconstitute the hematopoietic stem cell niche in the fetal liver. Some groups of stromal cells in stem cell niche could be responsible for producing growth factors to support stem cell expansion, for example, $CD34^{lo}CD133^{lo}$ cells, while certain cell types provide cell-cell contact with additional stimulating signals, for example, mesenchymal stem cells; other cell types potentially have the two overlapping features (Figure 1). These cells are referred to as stromal cells, which contribute soluble and/or membrane-bound signals to HSPCs as 'feeder cells', while those that are not involved in stem cell maintenance act as 'non-feeder cells'. Considering the complexity of the stem cell niche and the limited time and extent of stem cell expansion that researchers can currently achieve, the existing conditions for *ex vivo* stem cell expansion remain far from optimal. To achieve long-lasting robust *ex vivo* stem cell renewal and expansion, new factors should be explored to better mimic the hematopoietic stem cell niche. Theoretically, soluble and fixed components in a co-culture system can be obtained from the growth factors and the membrane-bound proteins that are present on feeder cells isolated from fetal livers, respectively. However, it is likely to be infeasible to continuously culture all potential feeder cell types in a single *ex vivo* culture system, considering the difficulty in obtaining and maintaining these cells for even a short period of time without changing their original features. Yong demonstrated that over time, primary $CD34^{lo}CD133^{lo}$ feeder cells rapidly change *in vitro*, leading to a dramatic decrease in the HSPC expansion efficiency when cultured for more than 7 days.[11] Thus, a feeder-free system in a 2D or a 3D format is likely to be more sustainable. In addition, although Yong detected the expression of a few key growth factors at the mRNA level, their protein translation, which is the real effector, could vary; the few known factors may only represent a small portion of the natural cocktail.

Can we overcome these issues and customize a feeder-free system with a combination of optimized soluble and immobilized factors? The following strategy to characterize and simplify the stem cell niche could be explored: first, gently disassociate the cellular network in the fetal liver, followed by an *ex vivo* 3D co-culture-based screening system and cell ablation to identify the essential cell components under the most robust conditions for the expansion of HSPCs. Proteomics and bioinformatics should subsequently be applied to identify the principal growth factors secreted and to characterize the membrane-bound receptor/ligand patterns formed between feeder cells and HSPCs. Finally, selected combinations should be verified in a novel feeder-free culture system with both soluble and anchored components.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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