Molecular interactome between HSCs and their niches

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Since hematopoietic stem cells (HSCs) were found to require a bone marrow (BM) habitat for long-term function, many studies have attempted to dissect key cellular and molecular interactions between HSCs and their BM microenvironments, or "HSC niches". In this issue of Blood, Mende N et al. provide a computational method to infer potential ligand-receptor interactions between murine hematopoietic stem and progenitor cells (HSPCs) and their niche-forming cells[1].

Niches formed by mesenchymal stem cells (MSCs) associated with endothelial cells were suggested a decade ago to critically regulate HSCs[2,3]. In the mouse, different genetic models have been used to mark MSCs and essentially agreed that these cells are an important source of key HSC factors[4,5]. However, a systematic comparison among HSC-niche cells identified using different strategies has been lacking until recently. Moreover, single-cell technologies have subsequently revealed that MSCs do not escape the large heterogeneity observed in other stem cell populations[6,7]. As a consequence, some discrepancies remain regarding the actual source and distribution of prominent HSC factors. The variable specificity, expression and penetrance across genetic models is a cause of debate. For example, combined deletion of essential HSC factors in multiple BM cell types (or the bulk of non-hematopoietic BM stroma) using constitutive Cre lines reduces HSCs[4,5] but cannot be compared with much smaller deletion in one cell population contained in the above and it has limited power to uncover specific HSC-niche cell interactions. The diversity of HSC niches is partly anatomical because different types of blood vessels and associated perivascular cells have been found in different BM regions, such as those in close apposition with the bone surface (endosteal) and those further away from bone (central). However, whether stromal or endothelial cells in different BM regions interact distinctly with HSCs has not been addressed in a systematic way.

In this issue of Blood, Mende N et al. undertake the impressive task of cross-comparing mesenchymal stromal cells and endothelial cells defined immunophenotypically and using genetic drivers in cell populations isolated from the central and the endosteal BM of mice[1]. Through their systematic assessment they confirm the overlap of HSC niche cell populations identified using different strategies – which had been suggested in previous studies – and identify interesting differences among cell populations harvested from endosteal and central BM (see figure). For instance, MSCs harvested from central or endosteal BM show distinct transcriptional profiles. Together with the reportedly higher resistance of endosteal MSCs to myeloablation and the described functions of some endosteal mesenchymal cells, such as N-cadherin$^+$ cells[8], these results add further evidence to the possibility that different BM niches might regulate steady-state vs. stress hematopoiesis.

As a major novel aspect, the authors develop a computational method to infer potential ligand-receptor interactions between niche cells and HSPCs based on mRNA expression levels. A ranking and matching algorithm assigns highly expressed ligands (either agonists or antagonists) in niche cells to putative receptors in HSPCs. Confirmation of key known interactions validates this method, which suggests potential novel interactions between HSPCs and their niches[1].

Previous studies have cross-compared the gene expression signatures of murine HSC-supporting and -non supporting stromal cell lines and have identified a modular network of paracrine signalling including most known and potential new HSC regulators[9].
Following the identification of new candidate interactive partners between HSCs and some niche cells, it would be interesting to functionally test them. The Wascow lab has already started this task by investigating the effect of several regulators on HSPCs *in vitro*[1].

Since the study by Mende et al. suggests differential interactions between HSPCs and $CD31^{hi}$ $CD144^{+}$ (putative arteriolar) endothelial cells or MSCs, it would be interesting to expand these analyses to other HSC-niche cells, such as $CD31^{lo}$ sinusoidal endothelial cells and megakaryocytes, for example. It is worth mentioning that this extensive cross-comparison of mouse models and prospectively identified HSC niche cells[1] has been performed at the cell population level. Following the development of HSC niche single-cell technologies[6,7], a natural extension of the current work would be applying these interactome algorithms to single cell transcriptomics and/or examining candidate interactome pathways using additional markers and single-cell RNAseq (or index sorting) to discriminate the specific cell types critically interacting with HSCs through novel pathways.

On a practical note, it would be very useful for the research community to develop a publicly available online resource to facilitate the application of the elegant mathematical algorithm developed by Höffer and colleagues[1] to the analyses of ligand-receptor interactions in other datasets by different laboratories. In that regard, other publicly available resources, such as the HSC niche database (http://stemniche.snv.jussieu.fr/), which allows to interrogate mRNA or miRNA expression in murine HSC-supporting stromal cells[9], or an interactive browser to interrogate gene expression in single BM vascular, perivascular and osteolineage cells (http://aifantislab.com/niche/)[6] have proven useful tools to investigate HSC niche cell transcriptomics.

Finally, future efforts in this area will need to include proteomics analysis since mRNA expression does not always correlate with protein abundance. This is particularly relevant for cytokine or chemokine receptors, which are often regulated by post-transcriptional mechanisms. Therefore, an important challenge for future studies is the extrapolation of candidate receptor-ligand interactions inferred from mouse transcriptomics to the human proteome. In this regard, age-associated alterations of HSPCs and their niches described in the human proteome resemble those previously found through mouse genetics[10], suggesting the potential translatability of these findings in general.

Figure Legend: In this issue of Blood, Mende et al.[1] use a variety of Cre lines targeting multiple non-hematopoietic cells of the BM microenvironment. They attempt to resolve previous discrepancies in the field whereby the overlap between niche cells targeted by different Cre lines was underestimated because only subsets among those Cre-targeted cells had been characterized. For that goal they use several reporters and perform RNAseq from immunophenotypically-identified HSPCs and mesenchymal stromal cells, endothelial cells

and preosteoblasts isolated from bone-associated (endosteal) BM or located further from bone (central). Matching top expressed ligands in niche cells with the most highly expressed receptors on HSPCs provides a candidate HSPC-niche interactome containing some old and many new potential HSPC regulators.

The author declares no conflict of interest.

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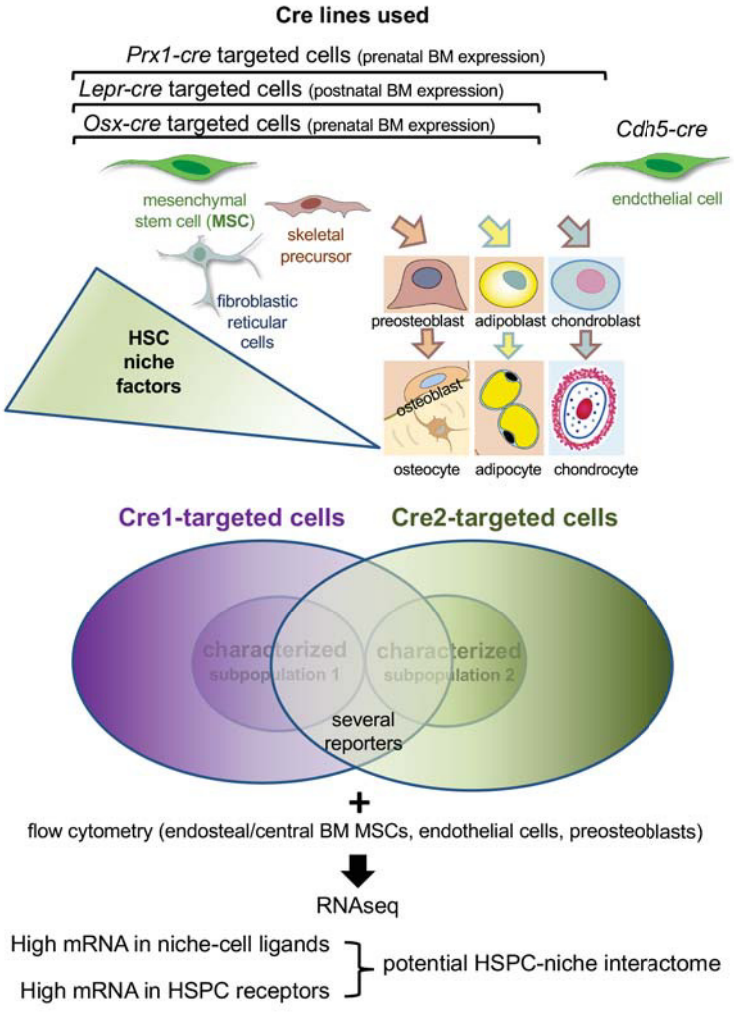
Cre lines used
Prx1-cre targeted cells (prenatal BM expression)
Lepr-cre targeted cells (postnatal BM expression)
Osx-cre targeted cells (prenatal BM expression)
Cdh5-cre
mesenchymal stem cell (MSC)
skeletal precursor
fibroblastic reticular cells
endcthelial cell
HSC niche factors
preosteoblast
adipoblast
chondroblast
osteoblast
osteocyte
adipocyte
chondrocyte
Cre1-targeted cells
Cre2-targeted cells
characterized subpopulation 1
characterized subpopulation 2
several reporters
+
flow cytometry (endosteal/central BM MSCs, endothelial cells, preosteoblasts)
RNAseq
High mRNA in niche-cell ligands
High mRNA in HSPC receptors
potential HSPC-niche interactome