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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Spatial Transcriptomics Reveals Distinct Hematopoietic Stem Cell Niches in Mouse Fetal Liver

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The hematopoietic stem cell (HSC) microenvironment, termed the niche, supports the proliferation, self-renewal, and differentiation abilities of HSCs. The definitive HSCs emerge from the hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region after E10.5, and then migrate to the fetal liver (FL) after E12.5 for expansion. After E18.5, HSCs migrate to the bone marrow where they reside for the postnatal stage and adulthood. Because the fetal liver is thought to be a harbor for the rapid expansion of HSCs, numerous studies have focused on the FL-HSC niche in the search for novel niche factors and niche cells that support HSC expansion. However, to our knowledge thus far, there have been no successes in translating the niche factors to a clinical application for the expansion of HSCs *ex vivo*. In this study, we use cutting-edge spatial transcriptomics to comprehensively investigate the transcriptomics and interactions between HSCs and the niche cells in the fetal liver. As a result, we have uncovered two distinct niches: the portal-vessel (PV) niche and the sinusoidal niche.

To understand the spatial distribution and interactions between FL-HSCs and niche cells, we introduced 2 spatial transcriptomic methods, Slide-seq (10uM resolution), and 10x Genomics Visium (55uM resolution), in our study on E14.5 and E16.5 mouse fetal liver. By integrating with single-cell RNA sequencing, we discovered the spatial transcriptomics of HSCs and potential niche cells, including hepatoblasts, endothelial cells, macrophages, megakaryocytes, and mesenchymal stromal cells (MSCs) in E14.5 and E16.5 mouse fetal liver. Interestingly, we found that MSCs and hepatoblasts were characterized by enriched N-cadherin expression. Both slide-seq and 10x Visium showed that the N-cadherin-expressing MSCs are enriched in the portal vessel area. Importantly, the majority of FL-HSCs are in close proximity to N-cadherin-expressing MSCs and endothelial cells, compared with other potential niche cells, indicating a supportive role of N-cadherin-expressing MSCs and endothelial cells in HSC maintenance. Subsequent CellPhoneDB (CPDB) analysis demonstrated that the N-cadherin-expressing MSCs are major niche-signaling senders with an enriched expression of niche factors and stemness pathway-related ligands. This finding is consistent with our previous finding that N-cadherin-expressing MSCs can maintain reserve HSCs in the adult bone marrow. To investigate the potential role of N-cadherin-expressing cells in supporting FL-HSCs, we generated an N-cadCreER;Cxcl12 and an N-cadCreER;Kitl mouse model to conditionally knockout the well-studied niche factors, Cxcl12 and Kitl (Scf), in N-cadherin-expressing cells. Interestingly, deletion of either Cxcl12 or Kitl through N-cad-CreERT induction resulted in the expansion of myeloid-biased FL-HSCs. Slide-seq further showed that depletion of Cxcl12 through N-cad-CreERT induction led to the repositioning of FL-HSCs from the PV region, which is enriched with MSCs, to the hepatic sinusoidal region, which lacks MSCs. These findings suggested the existence of distinct niches in the fetal liver: the PV niche is composed of N-cadherin $^{+}$ MSCs and peri-PV endothelial cells, and it maintains FL-HSCs with multilineage potential. In contrast, the sinusoidal niche preliminary consists of sinusoidal endothelial cells, hepatocytes, and hepatoblasts, which facilitates the proliferation of FL-HSCs but with a bias toward myeloid lineage. Previous studies suggested that the two forms of Kitl, soluble Kitl (sKitl) and membrane-bound Kitl (mKitl), may have different functions in supporting HSCs. Here, we discovered that mKitl is highly enriched in MSCs, whereas hepatoblasts are the major source of the sKitl. This finding suggests that mKitl may serve as the key niche factor that contributes to the homing and maintenance of FL-HSCs.

In summary, by using cutting-edge spatial transcriptomics, we uncovered the existence of distinct niches, the portal-vessel (PV) niche and the sinusoidal niche, in mouse fetal liver and the critical role of N-cadherin $^{+}$ MSCs in maintaining FL-HSCs and a role of hepatoblasts in promoting proliferation. The insight learned from this study enhances our understanding of the complex regulation of HSCs and opens new avenues for potential clinical applications.

Disclosures No relevant conflicts of interest to declare.

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