Fetal liver hematopoiesis is pivotal in embryonic development, driving essential blood cell production, this organ serves as a specialized niche orchestrating the differentiation, self-renewal, and proliferative expansion of hematopoietic stem and progenitor cells (HSPCs), ensuring the continuous supply of blood cells vital for fetal growth and development. This review outlines intrinsic and extrinsic regulatory mechanisms governing hematopoietic stem cells (HSCs) within the fetal liver niche, including transcription factors, signaling pathways, and cellular interactions. Recent advances in single-cell RNA sequencing and spatial transcriptomics have furthered our understanding of the fetal liver niche.

Exploring the fetal liver (FL) niche extends beyond its role in hematopoietic stem cell (HSC) expansion, offering valuable insights into the early development of the hematopoietic system and related diseases.

**Characteristics of FL HSCs**

The research challenges the traditional hierarchical model of hematopoiesis by showing independent generation of HSCs and progenitors at distinct sites, converging in the fetal liver, with minimal contribution of fetal HSCs to progenitor production in late gestation, indicating a stem cell-independent pathway for tissue growth. Additionally, it underscores the pivotal role of the transcription factor EVI1 in dictating the fate of pre-HSPCs, with EVI1hi cells mainly giving rise to HSCs.1

Recently, previously unnoticed heterogeneity in the immunophenotypic profile of FL-HSCs, highlighting the importance of relative biosynthetic dormancy, propensity for symmetric self-renewal, and differentiation latency in serially engraftable FL-HSCs, offering crucial insights for future ex vivo HSC amplification strategies.2

Unlike previous studies, a paper indicates that FL-HSCs exhibit a bias towards differentiation over symmetric cell division compared to neonatal and adult BM HSCs, suggesting that the HSC pool contributing to life-long hematopoiesis undergoes modest expansion during the FL stage of development.3

Another investigation revealed a subset of lymphoid-biased "developmentally-restricted HSCs" in the FL that exhibit long-term engraftment upon transplantation but do not contribute to adult hematopoiesis in situ4, consistent with earlier findings identifying FL-HSC populations with unique engraftment characteristics5.

Both HSC-intrinsic and environmental mechanisms have been proposed to explain such distinct properties of fetal

**Cell types in FL niche**

Over the years, researchers have extensively utilized components of the fetal liver (FL) niche in attempts to maintain and expand hematopoietic stem cells (HSCs) ex vivo 6-9. Notably, studies have highlighted the significant role of the fetal liver-derived stromal cell line AFT024 in supporting HSC expansion for extended periods, with demonstrated maintenance for at least 4-7 weeks8.

Zhang et al. discovered that CD3+ Ter119− stromal cells produce insulin-like growth factor 2 (IGF-2) to support long-term HSC expansion.9 Additionally, they found that angiopoietin-like 2 (Angptl2) and angiopoietin-like 3 (Angptl3) secreted by these stromal cells can increase HSC expansion by 24- and 30-fold in vitro, and these factors, along with IGF-2, can be supplemented as small molecules in the culture medium to promote HSC expansion.10

Findings indicate that hepatoblasts function as a niche for erythropoiesis via cytokine secretion of SCF and EPO11, while subsequent studies by a similar group reveal that Dlk1+ hepatic progenitors are the primary supportive cells for HSC expansion in the fetal liver6.

Hepatoblasts, mainly regulated by TGF-beta-1, primarily generate ECM factors crucial for HSC adhesion in the fetal liver niche.12

During fetal liver development, hepatoblasts emerge as primary sources of KITL, CXCL12, Epo, and IL-7, facilitating hematopoietic progenitor association from E12.5 to E14.5. However, by E18.5, as the hepatic cell composition shifts towards hepatocytes and cholangiocytes, levels of these factors decrease, aligning with the onset of bone marrow hematopoiesis.13

Macrophage PAR2 proteolytic signaling is crucial for definitive hematopoiesis and erythropoiesis in the fetal liver14. Additionally, it has been shown in zebrafish CHT that IFI30 expression in macrophages, closely associated with hematopoietic progenitors (CD117+), aids in reducing oxidative stress15.

Despite their low abundance in fetal liver cells, hepatic stellate cells express various hematopoietic cytokines and extracellular matrix mRNAs, potentially establishing a niche for liver hematopoiesis.16

Fetal liver endothelium governs erythropoiesis via mKitl secretion, modulated by Ezh2, marking the pioneering observation of an epigenetic factor's cell-extrinsic role in hematopoietic regulation.Top of Form17

The demonstration unveiled that fetal liver vascular niche endothelial cells (ECs) offer distinctive support for HSPC maturation and expansion compared to other organ-specific ECs, with a partial dependence on EC-derived WNT5A.18

Deletion of Scf from both endothelial and hepatic stellate cells resulted in the loss of nearly all HSCs, indicating the crucial role of these cells in creating the perisinusoidal vascular HSC niche in the developing liver through SCF production.19

EPCR+ HSCs are located in the Lyve1+ perisinusoidal niche in the mouse fetal liver, where the cytoprotective role of EPCR is crucial for maintaining these cells.19

Pericytes expressing Nestin and NG2 cluster around portal vessels, fostering a niche conducive to HSC expansion, and their quantities align with the fractal branching of portal vessels throughout development.20

Numerous studies emphasize the significance of FL EC and stromal cells in FL HSC expansion, yet only a handful delve into the functional relevance of hematopoietic cells, which comprise a substantial portion of the FL, as niche cells for FL HSCs.

Recent findings indicate that Jagged 1 expression in hematopoietic cells during the fetal stage is crucial for the functional potential of FL HSCs, highlighting the collaborative role of non-hematopoietic and hematopoietic cells in creating a distinctive niche for the expansion, survival, and functional maturation of FL HSCs.21

Hematopoietic progenitor cells (HPCs) and matured hematopoietic cells (HCs) release CCL17 and CCL22 ligands, which interact with CCR4 receptors expressed on fetal liver (FL) hematopoietic stem and progenitor cells (HSPCs), regulating their migration and retention within the FL niche through paracrine signaling.22

**Intrinsic regulation:**

FL-HSCs exhibit distinct metabolic profiles from adult HSCs, suggesting a pivotal metabolic switch crucial for maintaining HSC stemness during development, with FL-HSCs predominantly relying on mitochondrial respiration for energy.23,24

Sox17 and Lin28b transcription factors, abundant in fetal hematopoietic stem/progenitor cells (HSPCs) in both mice and humans, govern the distinctive behavior of fetal liver (FL) HSCs compared to adult bone marrow (BM) HSCs.Top of Form25,26

Our prior research revealed that pinpointing genes implicated in transcriptional regulation, exhibiting differential expression between expanding fetal liver (FL) HSCs and quiescent adult bone marrow (ABM) HSCs, unveils novel regulators of HSC function.Top of Form27

ESAM (Endothelial cell-selective adhesion molecule) plays distinct roles in the progression of definitive hematopoiesis within the fetal liver (FL), with particular significance observed in the development of adult-type erythropoiesis.28

MYSM1 is vital for normal HSC function and hematopoietic progression in the fetal liver.29

N-cad+ LSK cells in E12.5 fetal liver, co-localizing with sinusoidal endothelial cells (Lyve-1+ cells), showed increased long-term reconstitution (LTR) activity compared to N-cad− LSK cells, with activity declining at E15.5 and E18.5.30

Wnt signaling dynamics in murine fetal and adult HSPCs reveal a transition from Wnt/β-catenin-dependent signaling in fetal liver HSPCs to predominantly noncanonical Wnt/polarity signaling in adult HSPCs, with β-catenin selectively essential for fetal HSPC competitiveness post-transplant and conferring protection against oxidative stress.31

The identification of GPI-80 delineates a subset of self-renewing HSCs in humans, facilitating their tracking across various developmental niches. Molecular examination of GPI-80 HSPCs unveils novel regulatory mechanisms in highly self-renewing fetal HSCs, highlighting their utilization of pathways associated with leukocyte adhesion and migration to sustain self-renewal ability.32

Deletion of Notch1 TAD compromised purified FL HSC function in competitive reconstitution assays, revealing intrinsic reconstitution defects inherent to FL HSCs with the ΔTAD mutation.33

Optimal Notch signaling balance is crucial for fetal liver HSC maintenance; Bloc1s2 depletion results in heightened Notch signaling, increasing the frequency yet weakening the self-renewal capacity of FL HSCs.34

ATF4 enhances Angptl3 expression in niche cells, crucial for expanding and maintaining functional HSCs during fetal liver hematopoiesis.35

Extrinsic mechanism for FL HSC:

The significance of niche-derived antioxidant systems in reducing oxidative stress 15, alongside the role of exogenous bile acids in mitigating endoplasmic reticulum stress 36, both contributing to the protection of self-renewing hematopoietic stem cells (HSCs) during their regulated expansion within the fetal liver (FL) niche.

Our recent findings demonstrate that integrin signaling modulates HSC proliferation across developmental stages, including the fetal liver.37

**Mechanical property of FL niche**

The fetal liver niche offers not just cytokines but also unique mechanical properties conducive to HSC expansion, distinguishing it from the adult BM niche, although most research focuses on the latter. 38-40

Hydrogels matching the stiffness of fetal liver tissue, along with suspension culture, better maintained the number of LSK-SLAM cells compared to stiffer fibrin hydrogels.41

Exploring an ex vivo method that integrates the mechanistic properties and cellular composition of the fetal liver niche, including niche cell types crucial for HSC expansion and maintenance, could yield valuable insights.42

fetal liver (FL) cells exhibit superior engraftment potential compared to other hematopoietic sources. This heightened engraftment capacity, coupled with their therapeutic efficacy in experimental models of hematological disorders, underscores the promising clinical utility of FL cells in hematopoietic stem cell transplantation and regenerative medicine.43,44

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