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Fetal liver: an ideal niche for hematopoietic stem cell expansion

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Fetal liver (FL) is an intricate and highly vascularized hematopoietic organ, which can support the extensive expansion of hematopoietic stem cells (HSCs) without loss of stemness, as well as of the downstream lineages of HSCs. This powerful function of FL largely benefits from the niche (or microenvironment), which provides a residence for HSC expansion. Numerous studies have demonstrated that the FL niche consists of heterogeneous cell populations that associate with HSCs spatially and regulate HSCs functionally. At the molecular level, a complex of cell extrinsic and intrinsic signaling network within the FL niche cells maintains HSC expansion. Here, we summarize recent studies on the analysis of the FL HSCs and their niche, and specifically on the molecular regulatory network for HSC expansion. Based on these studies, we hypothesize a strategy to obtain a large number of functional HSCs via 3D reconstruction of FL organoid *ex vivo* for clinical treatment in the future.

fetal liver, niche, hematopoietic stem cell expansion, signaling network

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INTRODUCTION

Hematopoiesis is a highly hierarchical system, among which hematopoietic stem cells (HSCs) reside at the apex of the hierarchy. HSCs are capable of replenishing themselves and producing all mature blood lineages. During mammalian embryogenesis, the first *bona fide* HSC is generated from hemogenic endothelium in the aorta-gonad-mesonephros (AGM) region via endothelial-to-hematopoietic transition (EHT) (Bertrand et al., 2010; Boisset et al., 2010; Kissa and Herbomel, 2010). In addition, HSCs are also detected in the vitelline artery, umbilical artery, head and placenta (de Bruijn et al., 2000; Dzierzak and Robin, 2010; Li et al., 2012). Then, HSCs will migrate to FL through blood circulation. FL is the major site for embryonic HSC expansion (Ema and Nakauchi, 2000). Finally, HSCs will colonize into the thymus to commit lymphocyte specification, as well as home to the bone marrow (BM) to maintain lifelong hematopoiesis (Orkin and Zon, 2008).

The goal of hematopoiesis studies is not only to elucidate the developmental trajectory of blood cells, but also to provide useful insights for treatment of malignant blood diseases. For instance, HSC transplantation (HSCT) is considered the most effective method to cure malignant blood diseases (Felfly and Haddad, 2014; Zhao et al., 2016), but the limited source (mainly from BM) of suitable HSCs seriously impedes its wide application. Compared to BM, FL possesses a much more enhanced ability to support HSC expansion, which is an ideal model to investigate HSC expansion *in vitro*. Therefore, it is essential to fully understand the cellular and molecular mechanisms of HSC expansion in FL.

In this review, we firstly introduce FL HSC expansion and the FL niche, including a complex of components of niche cells and their association with HSCs. Next, we summarize signaling pathways involved in FL HSC expansion. Finally,

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we propose several open questions to be addressed in this field.

HEMATOPOIETIC CELL EXPANSION IN FL

During embryogenesis, FL acts as a major site for HSC expansion. Through transplantation assays, Ema and Nakauchi found that HSC activities cannot be detected in FL until E12 (Ema and Nakauchi, 2000). Then, FL HSCs experience a dramatic expansion process (Mikkola and Orkin, 2006). Eventually, HSCs will expand up to the maximal quantity at E16.5; thereafter, the quantity of HSCs will decrease due to their mobilization out of FL (Gekas et al., 2005; Morrison et al., 1995). Moreover, several groups found that the immature HSC precursors (pre-HSCs), which are generated in the AGM region (Medvinsky and Dzierzak, 1996), will colonize into FL for maturation. Combined with transplantation assays, limiting dilution analysis and cells/organ culture, they demonstrated that a number of pre-HSCs will rapidly mature into definitive HSCs and form a large HSC pool in FL. This may explain why FL has a dramatic increase of HSCs at E12 (Kieusseian et al., 2012; Rybtsov et al., 2016).

In addition to HSC expansion, FL is also a site for expansion of downstream lineage cells of HSCs. The erythroid progenitors exhibit robust expansion during E12.5 to E18.5 in FL (Porayette and Paulson, 2008). Moreover, the proper expansion of myeloid and lymphoid lineage cells is very important for tissue homeostasis. Functional natural killer (NK) cells are generated by NK-cell progenitors (NKP) which emerge in E13.5 and expand during E14.5 and E15.5 in FL (Tang et al., 2012). Additionally, macrophages also show a powerful expansion in FL during E12.5–E14.5 and expand up to the peak at E14.5 (Hoeffel et al., 2015). Taken together, FL is a major site for the expansion of HSCs and their derivatives during embryogenesis.

THE PURIFICATION OF FL HSCS

AA4.1, a type of cell-surface glycoprotein, is the first cell surface marker used to isolate HSCs from mouse FL and AA4.1$^+$ cell population occupies 0.5%–1% FL cells (Jordan et al., 1990). Meanwhile, Weissman and his colleagues also purified mouse FL HSCs using combinatorial markers. They firstly found that mouse FL HSCs are enriched in the Thy-1^{lo} Lin$^-$ Sca-1$^+$ cell population (occupied 0.05% FL cells) (Ikuta et al., 1990). Then, c-Kit, Mac-1 and CD4 were proposed to purify Thy-1^{lo} Lin$^-$ Sca-1$^+$ FL HSCs (Ikuta and Weissman, 1992; Morrison et al., 1995). Besides, 7% of Lin$^-$ Sca-1$^+$ c-Kit$^+$ (LSK) AA4.1$^+$ cell population expresses Tie-2 that is the receptor of tyrosine kinase, and LSK AA4.1$^+$ Tie-2$^+$ cells have the long-term multilineage reconstitution ability (Hsu et al., 2000). In 2006, Morrison group enhanced the purification of mouse FL HSCs using signaling lymphocyte activation molecule (SLAM) family markers, including CD150 and CD48. They found that SLAM family markers have the similar expression pattern in FL HSCs compared with adult BM HSCs. The efficiency of purification with CD150$^+$ CD48$^-$ Sca-1$^+$ Lin$^-$ Mac-1$^+$ combination is significantly improved (Kim et al., 2006), and now it becomes a commonly used method to obtain purified FL HSCs. Additionally, EPCR$^+$ FL LSK and Flt-3$^-$ FL cells also possess HSC characteristics (Iwasaki et al., 2010; Zeigler et al., 1994). Interestingly, there are also some markers that are differently expressed between FL HSCs and BM HSCs in mice (Baumann et al., 2004; Kim et al., 2005). For instance, CD144 (also known as Cdh5), a classic cadherin on vascular endothelial cells, has been demonstrated to be transiently expressed in FL HSCs from E13.5 to E16.5. This finding also implies the close relationship between vascular endothelial cells and hematopoietic cells (Kim et al., 2005). Collectively, these studies contribute to purifying authentic HSCs from FL, and isolation of purified HSCs is the prerequisite to investigate the expansion of HSCs.

THE FL NICHE MODULATES HSC EXPANSION

The number of primary HSCs (from FL or BM) is very limited, and these isolated HSCs need to be cultured *in vitro* for both basic research and clinic treatment (i.e. HSCT). Hence, it is crucial to comprehend the detailed process of HSC expansion *in vivo*. Given that FL HSCs undergo rapid expansion and still maintain complete stemness, the FL niche serves as a paradigm for HSC expansion studies.

Mounting evidence has demonstrated that hepatic progenitors, stromal cells, pericytes and endothelial cells in mouse FL are the crucial niche cells for the expansion of HSCs (Khan et al., 2016; Swain et al., 2014). Zhang et al. identified a specific cell population: CD3$^+$ Ter119$^-$ stromal cells which can yield insulin-like growth factor 2 (IGF-2) to support long-term HSC expansion (Zhang and Lodish, 2004). Moreover, they also found that angiopoietin-like 2 (Angptl2) and angiopoietin-like 3 (Angptl3) secreted by the CD3$^+$ FL stromal cells can facilitate 24- and 30-fold expansion of HSCs after culture *in vitro*. More importantly, Angptl2, Angptl3 and IGF-2 can be added into the medium as small molecules to promote HSC expansion (Zhang et al., 2006). Furthermore, a co-culture system using SCF$^+$ DLK$^+$ FL hepatic progenitors has been developed, and it has been shown to be able to support the expansion of HSCs during *in vitro* culture (Chou and Lodish, 2010). Specifically, the SCF$^+$ DLK$^+$ cells can secret growth factors, including stem cell factor (SCF), IGF-2, Angptl3, and C-X-C motif chemokine

ligand 12 (CXCL12), which are important for mouse FL HSC expansion. Moreover, Zhao et al. found that ATF4-expressed FL stromal cells also have the positive impact on the expansion of FL HSCs (Zhao et al., 2015).

Interestingly, the group led by Paul Frenette systematically analyzed the spatial association between FL HSCs and niche cells, and found that FL HSCs had a close physical association with portal vessel and Nestin$^+$ NG2$^+$ pericytes, and about 40% FL HSCs reside within 20 μm of portal vessels (Gao et al., 2018; Khan et al., 2016). Besides, Nestin$^+$ pericytes can produce growth factors such as SCF, secreted phosphoprotein 1 (Spp1), IGF-2, Angptl2 and CXCL12 to support HSC expansion (Figure 1). They also dissected the intricate vascular network and generated the three-dimensional (3D) reconstruction of FL vasculature. In addition, they found that the expression of growth factors (SCF, Angptl2, IGF-2), presents similar levels from E12.5 to E14.5, but the vascular structure extends obviously accompanied with the HSC expansion in FL. Using this model, they further emphasized that the extension of niche structure may govern the HSC expansion.

THE NETWORK OF SIGNALING PATHWAYS REGULATES HSC EXPANSION

To deeply elucidate why FL can provide an extremely suitable niche for HSC expansion, it is necessary for us not only to identify the niche cells surrounding HSCs, but also to define how exactly the complicated network of signaling pathways in FL niche impacts HSC expansion.

Growth factors

Accumulating data show that HSCs can be maintained *ex vivo* with addition of combinations of growth factors, including SCF, Flt-3 ligand and interleukin family (Metcalf, 2008). SCF is a widely-used growth factor to promote HSC expansion. Its receptor, c-Kit, resides at the membrane of stem cells (Flanagan and Leder, 1990; Williams et al., 1990). The binding of SCF to its receptor will trigger the downstream signaling cascades, such as MEK/ERK and PI3K/Akt kinase signaling pathways (Edling and Hallberg, 2007), and further upregulate the expression of expansion-associated regulators (Munugalavadla et al., 2005; Xie et al., 2014). Additionally, SCF/c-Kit signaling pathway is also engaged in sustaining the HSC survival via anti-apoptosis pathway (Li and Johnson, 1994). Zayas et al. revealed that tr-Kit, a truncated form of c-Kit, is restrictedly expressed in cell populations enriched for HSCs and MPPs of FL and BM, and exerts functions in a SCF-dependent manner. In view of the specific expression of tr-Kit, more phenotypic HSCs can be isolated for *in vitro* culture (Zayas et al., 2008). Furthermore, the multiple origins of SCF have been well demonstrated in the adult BM niche in which different cell-derived SCF plays distinct roles in regulating HSCs (Ding et al., 2012; Zhou et al., 2017). Given that the niche components of FL are distinct from BM as well as their corresponding HSCs (Crane et al., 2017; Morrison and Scadden, 2014; Swain et al., 2014), we speculate that the similar strategy can be adopted to uncover the exact mechanism of how a particular niche cell-specific SCF regulates FL HSC expansion.

Previous studies indicated that HSCs are able to expand when co-cultured with FL cells in medium (Chou and Lodish, 2010). Further analyses unravel that growth factors produced by the co-cultured cells are the major cause of HSC expansion. IGF-2 is generated by mouse FL CD3$^+$ Ter119$^-$ cell population and can promote HSC expansion. Mechanistically, a high concentration of IGF-2 binds to the receptors, IGF1R, IR and IGF2R, and activates the downstream signaling cascades. On the one hand, IGF-2, along with SCF and thrombopoietin (TPO), activates mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI-3K) pathways to facilitate HSC expansion. On the other hand, IGF-2 may prevent HSCs from apoptosis (Zhang and Lodish, 2004). Moreover, FL CD3$^+$ cells are also reported to secrete Angptl2 and Angptl3, which could stimulate HSC expansion. Thus, the growth factors combination can achieve a higher efficiency to increase the number of HSCs, but the downstream signaling pathways triggered by Angptl2/3 remain to be discovered (Zhang et al., 2006). Recently, the receptor of ANGPTL proteins, leukocyte immunoglobulin (Ig)-like receptor B2 (LILRB2), was identified in human and is able to support HSC expansion *in vitro* (Zheng et al., 2012). Furthermore, Lin et al. showed that ANGPTL2 facilitates the NOTCH receptor cleavage through binding of LILRB2, and then activates intranuclear target genes, including *MYC* and *RUNX1*. The activation of MYC expression could explain why HSCs expand *ex vivo* with the addition of Angptl2 (Lin et al., 2015). However, it was still unclear which upstream regulators can modulate the synthesis of Angptl proteins. To this end, Cheng and his colleagues demonstrated that ATF4 has a critical role in FL to support HSC expansion and further analysis indicates that ATF4 can regulate *Angptl3* transcription through extrinsic regulation to maintain HSC expansion (Zhao et al., 2015).

Notch signaling

Hackney et al. performed the first global analysis to characterize mouse FL stromal-derived signaling pathways at the molecular level and described the first molecular profile of stroma cell line, the well-known stroma cell line, AFT024. A number of novel candidate signaling molecules were identified; among them, Notch was verified to regulate HSC maintenance and homeostasis (Coskun and Hirschi, 2010;

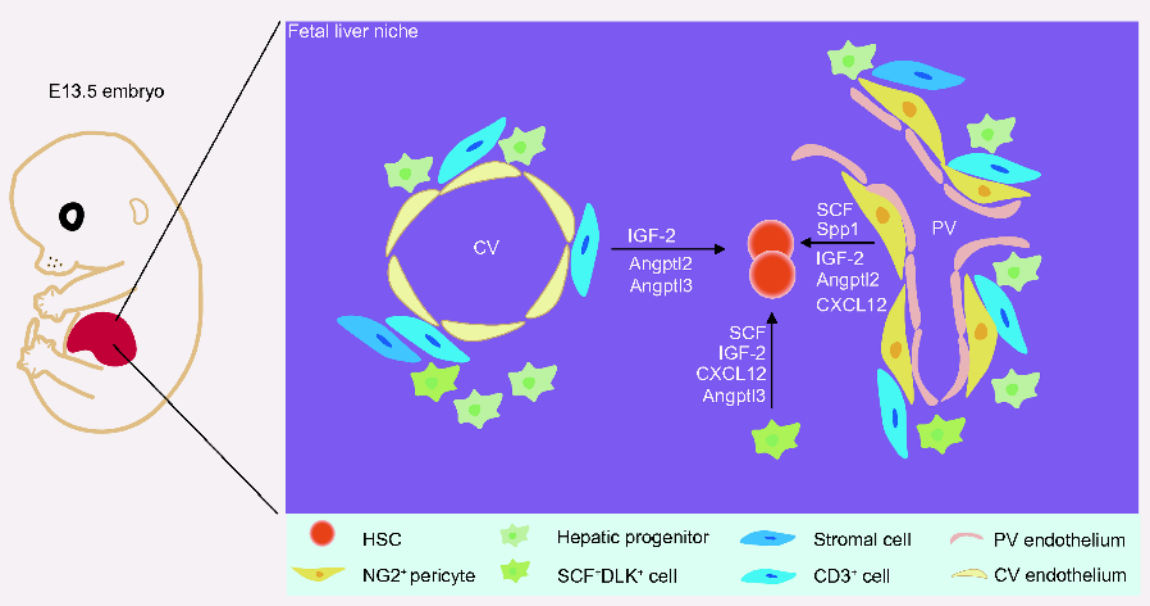

Figure 1 (Color online) The fetal liver niche regulates HSC expansion. A schematic diagram of the fetal liver (FL) niche. Vascular endothelial cells, pericytes, stromal cells and hepatic progenitors are the major niche cells that can modulate HSC extensive expansion through producing growth factors, such as IGF-2, Angptl2/3, SCF and CXCL12. These cell-extrinsic factors will trigger intracellular signaling pathways and stimulate HSC expansion. Angptl2/3, angiopoietin-like 2/3;. CV, central vein; PV, portal vessel.

Hackney et al., 2002). Notch signaling pathway is mediated by the cell-cell contact and is engaged in diverse biological processes. In mouse FL, He et al. revealed that the balanced level of Notch signaling regulated by BLOS2 is indispensable for HSC homeostasis. Over-activated Notch signaling will augment the frequency of HSCs, whereas disturb their self-renewal ability (He et al., 2017). Besides, Notch1/Rbpj/Maml trimolecular transcription complex is involved in mouse FL HSCs homeostasis. It implies that the appropriate threshold of signaling activation and dynamic regulation are critical for HSC homeostasis and maintenance (Gerhardt et al., 2014). Moreover, Cited2 (cAMP-responsive element binding protein), as a transcriptional modulator, plays an important role in mouse FL HSC expansion as well as in the maintenance of hematopoietic homeostasis through hypoxic response and Notch signaling (Chen et al., 2007).

Epigenetic regulation

Epigenetic regulation is an indispensable mechanism to regulate HSC expansion. BAF250a, a component of the SWI/SNF-BAF chromatin remodeling complex, plays an extrinsic role to establish and maintain definitive HSCs in mouse FL (Krosl et al., 2010). BRPF1 can regulate multipotent genes expression, including Gfi1, Hoxa9, and Gata3, through acetylation of histone H3 at lysine 23 and plays an essential role in definitive hematopoiesis in FL (You et al., 2016). EZH2, a key member of PRC2, is required for HSCs maintenance in mouse FL through regulating expression of cell cycle genes in a methyltransferase-dependent manner (Mochizuki-Kashio et al., 2011). Although the detailed molecular mechanisms are different individually, all these signaling pathways mediated by epigenetic modifiers can guarantee that HSCs have a stable and normal maturation, expansion, differentiation and migration in FL.

COMPREHENSIVE OMICS PROFILING OF FL AND HSC ONTOGENY

To systematically understand the underlying mechanisms of how FL supports HSC expansion, various omics approaches have been applied to study mouse FL HSCs and their niche at both transcriptional and protein levels.

Zhang group firstly performed a systemic analysis of mouse FL gene expression via high-density oligonucleotide microarrays (Li et al., 2009). The results showed that FL at different developmental stages present different gene expression pattern. Specifically, E11.5–E14.5 FL highly expresses cell-cycle and cell division genes, such as mRNA processing, transcription, ribosome biogenesis and translation. E14.5–E18.5 FL shows high expression of genes associated with FL function, such as biosynthetic process and signal transduction, while the genes involved in innate immune response are enriched in neonatal FL. They showed that the gene expression of FL presents a dynamic variation, which will helps us understand the mechanism of FL ontogeny (Li et al., 2009). Furthermore, Daley and his colleagues

performed the transcriptome analysis using HSCs derived from different hematopoietic sites in mice, including AGM, placenta, YS, ESCs, FL (E12.5–14.5) and BM (McKinney-Freeman et al., 2012). They found that embryonic HSCs can be divided into three groups, including YS-Like, specifying HSC and definitive HSCs. Among them, FL HSCs belong to the group of definitive HSCs and highly express expansion-related genes, whereas *Fos* and *Fosb* that negatively control cell cycle progression are downregulated in FL HSCs. The detailed characterization of transcriptional landscape of FL and HSCs ontogeny is essential to understand the mechanism of HSC expansion in FL (McKinney-Freeman et al., 2012).

Additionally, systemic proteomics analysis is crucial for uncovering the functional effects in FL HSC expansion. Hansson group comprehensively characterized the proteome of mouse FL HSCs using the spectrometry-based quantitative proteomics method (Jassinskaja et al., 2017). As a result, they found that anabolic signaling pathways are enriched in FL HSCs, such as glycogen synthesis and lipid synthesis. This finding suggests that high level of metabolism may contribute to the rapid expansion of HSCs. Meanwhile, FL HSCs show low level of proteins involved in antioxidative process, which implies that FL HSCs are more sensitive to high level of ROS (Jassinskaja et al., 2017).

Single cell RNA sequencing makes it possible to construct a precise cell atlas for any tissues/organs and even a whole organism. Very recently, Guo group constructed the E14.5 FL cell atlas using 3,730 single cells (Han et al., 2018). Based on the atlas, they detected 3,124 genes, identified 11 cellular clusters, and the complex cellular component of FL was revealed through this atlas, including the cell classification, cell proportion of each cellular cluster and the signature genes of each cell type. The construction of cell atlas at the organ level improves our understanding about the FL cell types, as well as their function associated with HSCs.

Taken together, all these omics approaches provide deep insights into the transcriptional landscape and proteomics analysis of HSCs and FL niche cells. This will help us comprehensively understand FL and HSCs ontogeny, and uncover the underlying mechanism of HSC expansion in FL.

REMAINING QUESTIONS

The studies on FL as being a potent hematopoietic organ, began in the 1960s with the demonstration of its reconstitution ability (Kubanek et al., 1969). In the past decades, many fancy and highly sensitive techniques have been applied to dissect and map the FL niche, the spatial location of HSCs, as well as key signaling pathways that regulate HSC expansion. In addition, several outstanding questions remain to be addressed: (i) Given that HSCs expand rapidly in FL but maintain quiescent in BM, it is unknown which factors trigger this transition; (ii) how to reconstruct the FL organoid *in vitro* using the advanced techniques to acquire sufficient functional HSCs for clinical use.

FL HSCs maintain active expansion, whereas BM HSCs generally keep quiescence. Two important hematopoietic organs endow HSCs distinct characteristics. Therefore, it is intriguing to uncover the underlying mechanism of how these differences occur. The BM niche has been convincingly demonstrated to be essential for the maintenance of BM HSCs. Through direct confocal imaging and functional assays, BM HSCs are illustrated residing at a specialized niche. Morrison group found that about 85% HSCs are associated with sinusoids in mouse BM (Acar et al., 2015). Moreover, Frenette group demonstrated that arteriolar niche plays an important role in maintaining mouse BM HSC quiescence (Kunisaki et al., 2013). These studies suggest that BM HSCs associate with specific vascular niche to maintain their characteristics. In contrast, FL HSCs get close to the portal vessel to sustain their activity (Khan et al., 2016). Collectively, different niches may contribute to different characteristics of HSCs. Moreover, although both BM HSCs and FL HSCs can respond to growth factors, such as SCF and CXCL12, the different intracellular signaling pathway triggered by the same growth factors may result in different cell behavior (Boulais and Frenette, 2015; Crane et al., 2017; Morrison and Scadden, 2014).

In addition to the cell-extrinsic differences, there are also cell-intrinsic differences between FL and BM HSCs. Viewing from the phenotypic difference, FL HSCs exhibit higher expression of the genes involved in oxidative phosphorylation, cell expansion and the citric acid cycle to fuel the ability of extensive expansion than BM HSCs (Manesia et al., 2015), Furthermore, FL HSCs possess more mitochondria compared with BM HSCs (Manesia et al., 2015; McKinney-Freeman et al., 2012). The dynamic metabolic demands are responsible for HSC expansion and homeostasis. Regarding the functional differences, FL HSCs exhibit faster rate of expansion and higher reconstruction ability in transplanted recipient mice than BM HSCs. Moreover, the proportion of myeloid lineage output of transplanted FL HSCs in recipients show higher than that of BM HSCs (Arora et al., 2014; Harrison et al., 1997; Szilvassy et al., 2001). Collectively, these differences may be attributed to, at least part of, cell-intrinsic factors rather than the niche to drive FLHSC expansion.

Systematic investigation of an organ is extremely crucial for a full understanding of the regulatory mechanisms that maintain organ function and homeostasis. The complex spatial structure of FL, as well as low efficient methods in the past, hampered our abilities to fully characterize the FL. However, with the advancement of interdisciplinary research, more advanced approaches are developed to gain a

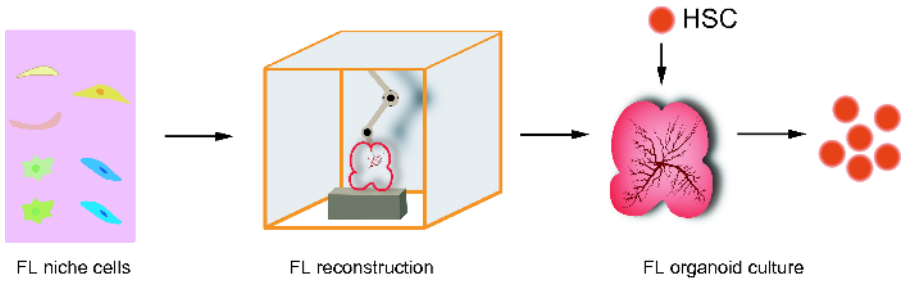

Figure 2 (Color online) Three-dimensional reconstruction of the FL organoid for HSC expansion. A schematic diagram of HSC expansion via an artificial FL organoid. Through mimicking the structure of FL niche *in vivo*, the FL organoid is generated with the help of three-dimensional reconstruction techniques. The artificial FL organoid will provide an ideal platform for HSC expansion.

deeper insight into the spatial structure of niche and HSC expansion in FL. The low-input and high-throughput omics analysis represented by single-cell RNA-sequencing (Treutlein et al., 2014), have helped redefine the previously unknown cell-types, which have been largely ignored in cell-population based studies. The more cell types are identified, the more definitive it is for us to fully comprehend the FL cells atlas. The current single-cell expression profiling analysis includes Drop-sequencing (Macosko et al., 2015), CEL-sequencing (Grün et al., 2014; Grün and van Oudenaarden, 2015), and Microwell-sequencing (Han et al., 2018). However, the limitation of these methods is to dissect FL cells at two-dimensional (2D) level, whereas the *in situ* spatial information remains elusive. Interestingly, GEO-sequencing and Tomo-sequencing have been developed to investigate the spatial pattern of gene expression. The spatial genome-wide transcriptome analysis, combined with the positional information of specific cells, contribute to 3D visualization of a certain tissue or organ (Chen et al., 2017; Peng et al., 2016). These excellent techniques derived from a crosstalk of multiple disciplines will make it possible to establish the spatial network of signaling pathways and spatial vascular network within the FL niche, and even for 3D reconstruction of a whole FL organ.

The bottleneck for successful artificial FL organoid is the functional vascular network and the precise orchestration between FL niche cells. Interestingly, an important advancement in tissue engineering field is the design of bio-scaffold. The extracellular matrix (ECM), including the components of structural and functional proteins, has become the popular three-dimensional scaffold for tissue reconstruction (Badylak, 2002). Moreover, Baptista et al. developed a new bio-scaffold for bioengineered liver via a decellularization process, and a significant advance of this bio-scaffold is to protect the macrovascular skeleton and allow the niche cells easily enter (Baptista et al., 2011). Liver cells, endothelial cells and stromal cells can enter the scaffold and reseed the vascular network to realize the functional artificial FL organoid. Besides, several studies suggested that the recapitulation of the dynamic cellular organization and cellular interaction of human FL cells during the organogenesis would stimulate the cellular self-organizing ability to promote the generation of organoid (Szpinda et al., 2015; Takebe et al., 2012). Based on these studies, we hypothesize that the artificial FL organoid takes advantage of the decellularized bio-scaffold, primitive niche cells and optimized culture condition to precisely mimic organogenesis, therefore the artificial FL organoid will play an important role in HSC expansion and drug discovery.

Taken together, all these techniques greatly improve our understanding on the nature of FL HSC expansion *in vivo* and are proposed to help produce a large number of functional HSCs for clinical applications (Figure 2).

CONCLUSION

In summary, we review the current knowledge about the FL niche, which is engaged in HSC expansion. With the help of novel techniques, scientists revealed the spatial structure of vasculature and its close association with FL HSCs (Khan et al., 2016). These evolving views and emerging techniques will greatly facilitate our understanding on the crosstalk between the FL niche and HSC expansion, as well as help to bridge the gap from bench to bedside. For example, further investigation is required to recapitulate the FL organ *in vivo* to establish a FL-like culture condition, and even to establish an artificial FL organoid, for HSC expansion in clinical applications.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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Acar, M., Kocherlakota, K.S., Murphy, M.M., Peyer, J.G., Oguro, H., Inra,

C.N., Jaiyeola, C., Zhao, Z., Luby-Phelps, K., and Morrison, S.J. (2015). Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. Nature 526, 126–130.
Arora, N., Wenzel, P.L., McKinney-Freeman, S.L., Ross, S.J., Kim, P.G., Chou, S.S., Yoshimoto, M., Yoder, M.C., and Daley, G.Q. (2014). Effect of developmental stage of HSC and recipient on transplant outcomes. Dev Cell 29, 621–628.
Badylak, S.F. (2002). The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol 13, 377–383.
Baptista, P.M., Siddiqui, M.M., Lozier, G., Rodriguez, S.R., Atala, A., and Soker, S. (2011). The use of whole organ decellularization for the generation of a vascularized liver organoid. Hepatology 53, 604–617.
Baumann, C.I., Bailey, A.S., Li, W., Ferkowicz, M.J., Yoder, M.C., and Fleming, W.H. (2004). PECAM-1 is expressed on hematopoietic stem cells throughout ontogeny and identifies a population of erythroid progenitors. Blood 104, 1010–1016.
Bertrand, J.Y., Chi, N.C., Santoso, B., Teng, S., Stainier, D.Y.R., and Traver, D. (2010). Haematopoietic stem cells derive directly from aortic endothelium during development. Nature 464, 108–111.
Boisset, J.C., van Cappellen, W., Andrieu-Soler, C., Galjart, N., Dzierzak, E., and Robin, C. (2010). *In vivo* imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature 464, 116–120.
Boulais, P.E., and Frenette, P.S. (2015). Making sense of hematopoietic stem cell niches. Blood 125, 2621–2629.
Chen, J., Suo, S., Tam, P.P., Han, J.D.J., Peng, G., and Jing, N. (2017). Spatial transcriptomic analysis of cryosectioned tissue samples with Geo-seq. Nat Protoc 12, 566–580.
Chen, Y., Haviernik, P., Bunting, K.D., and Yang, Y.C. (2007). Cited2 is required for normal hematopoiesis in the murine fetal liver. Blood 110, 2889–2898.
Chou, S., and Lodish, H.F. (2010). Fetal liver hepatic progenitors are supportive stromal cells for hematopoietic stem cells. Proc Natl Acad Sci USA 107, 7799–7804.
Coskun, S., and Hirschi, K.K. (2010). Establishment and regulation of the HSC niche: roles of osteoblastic and vascular compartments. Birth Defects Res Part C Embryo Today Rev 90, 229–242.
Crane, G.M., Jeffery, E., and Morrison, S.J. (2017). Adult haematopoietic stem cell niches. Nat Rev Immunol 17, 573–590.
de Bruijn, M.F.T.R., Speck, N.A., Peeters, M.C.E., and Dzierzak, E. (2000). Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. EMBO J 19, 2465–2474.
Ding, L., Saunders, T.L., Enikolopov, G., and Morrison, S.J. (2012). Endothelial and perivascular cells maintain haematopoietic stem cells. Nature 481, 457–462.
Dzierzak, E., and Robin, C. (2010). Placenta as a source of hematopoietic stem cells. Trends Mol Med 16, 361–367.
Edling, C.E., and Hallberg, B. (2007). c-Kit—A hematopoietic cell essential receptor tyrosine kinase. Int J Biochem Cell Biol 39, 1995–1998.
Ema, H., and Nakauchi, H. (2000). Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. Blood 95, 2284–2288.
Felfly, H., and Haddad, G.G. (2014). Hematopoietic stem cells: potential new applications for translational medicine. J Stem Cells 9, 163–197.
Flanagan, J.G., and Leder, P. (1990). The kit ligand: A cell surface molecule altered in steel mutant fibroblasts. Cell 63, 185–194.
Gao, X., Xu, C., Asada, N., and Frenette, P.S. (2018). The hematopoietic stem cell niche: from embryo to adult. Development 145, dev139691.
Gekas, C., Dieterlen-Lièvre, F., Orkin, S.H., and Mikkola, H.K.A. (2005). The placenta is a niche for hematopoietic stem cells. Dev Cell 8, 365–375.
Gerhardt, D.M., Pajcini, K.V., D'altri, T., Tu, L., Jain, R., Xu, L., Chen, M. J., Rentschler, S., Shestova, O., Wertheim, G.B., et al. (2014). The Notch1 transcriptional activation domain is required for development and reveals a novel role for Notch1 signaling in fetal hematopoietic stem cells. Genes Dev 28, 576–593.
Grün, D., Kester, L., and van Oudenaarden, A. (2014). Validation of noise models for single-cell transcriptomics. Nat Methods 11, 637–640.
Grün, D., and van Oudenaarden, A. (2015). Design and analysis of single-cell sequencing experiments. Cell 163, 799–810.
Hackney, J.A., Charbord, P., Brunk, B.P., Stoeckert, C.J., Lemischka, I.R., and Moore, K.A. (2002). A molecular profile of a hematopoietic stem cell niche. Proc Natl Acad Sci USA 99, 13061–13066.
Han, X., Wang, R., Zhou, Y., Fei, L., Sun, H., Lai, S., Saadatpour, A., Zhou, Z., Chen, H., Ye, F., et al. (2018). Mapping the mouse cell atlas by microwell-Seq. Cell 172, 1091–1107.e17.
Harrison, D.E., Zhong, R.K., Jordan, C.T., Lemischka, I.R., and Astle, C. M. (1997). Relative to adult marrow, fetal liver repopulates nearly five times more effectively long-term than short-term. Exp Hematol 25, 293–297.
He, Q., Gao, S., Lv, J., Li, W., and Liu, F. (2017). BLOS2 maintains hematopoietic stem cells in the fetal liver via repressing Notch signaling. Exp Hematol 51, 1–6.e2.
Hoeffel, G., Chen, J., Lavin, Y., Low, D., Almeida, F.F., See, P., Beaudin, A.E., Lum, J., Low, I., Forsberg, E.C., et al. (2015). C-Myb^{+} erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunity 42, 665–678.
Hsu, H.C., Ema, H., Osawa, M., Nakamura, Y., Suda, T., and Nakauchi, H. (2000). Hematopoietic stem cells express Tie-2 receptor in the murine fetal liver. Blood 96, 3757–3762.
Ikuta, K., Kina, T., MacNeil, I., Uchida, N., Peault, B., Chien, Y., and Weissman, I.L. (1990). A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. Cell 62, 863–874.
Ikuta, K., and Weissman, I.L. (1992). Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation.. Proc Natl Acad Sci USA 89, 1502–1506.
Iwasaki, H., Arai, F., Kubota, Y., Dahl, M., and Suda, T. (2010). Endothelial protein C receptor-expressing hematopoietic stem cells reside in the perisinusoidal niche in fetal liver. Blood 116, 544–553.
Jassinskaja, M., Johansson, E., Kristiansen, T.A., Åkerstrand, H., Sjöholm, K., Hauri, S., Malmström, J., Yuan, J., and Hansson, J. (2017). Comprehensive proteomic characterization of ontogenic changes in hematopoietic stem and progenitor cells. Cell Rep 21, 3285–3297.
Jordan, C.T., McKearn, J.P., and Lemischka, I.R. (1990). Cellular and developmental properties of fetal hematopoietic stem cells. Cell 61, 953–963.
Khan, J.A., Mendelson, A., Kunisaki, Y., Birbrair, A., Kou, Y., Arnal-Estapé, A., Pinho, S., Ciero, P., Nakahara, F., Ma'ayan, A., et al. (2016). Fetal liver hematopoietic stem cell niches associate with portal vessels. Science 351, 176–180.
Kieusseian, A., Brunet de la Grange, P., Burlen-Defranoux, O., Godin, I., and Cumano, A. (2012). Immature hematopoietic stem cells undergo maturation in the fetal liver. Development 139, 3521–3530.
Kim, I., He, S., Yilmaz, O.H., Kiel, M.J., and Morrison, S.J. (2006). Enhanced purification of fetal liver hematopoietic stem cells using SLAM family receptors. Blood 108, 737–744.
Kim, I., Yilmaz, O.H., and Morrison, S.J. (2005). CD144 (VE-cadherin) is transiently expressed by fetal liver hematopoietic stem cells. Blood 106, 903–905.
Kissa, K., and Herbomel, P. (2010). Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature 464, 112–115.
Krosl, J., Mamo, A., Chagraoui, J., Wilhelm, B.T., Girard, S., Louis, I., Lessard, J., Perreault, C., and Sauvageau, G. (2010). A mutant allele of the Swi/Snf member BAF250a determines the pool size of fetal liver hemopoietic stem cell populations. Blood 116, 1678–1684.
Kubanek, B., Rencricca, N., Porcellini, A., Howard, D., and Stohlman, F. (1969). The pattern of recovery of erythropoiesis in heavily irradiated mice receiving transplants of fetal liver. Exp Biol Med 131, 831–834.
Kunisaki, Y., Bruns, I., Scheiermann, C., Ahmed, J., Pinho, S., Zhang, D., Mizoguchi, T., Wei, Q., Lucas, D., Ito, K., et al. (2013). Arteriolar niches maintain haematopoietic stem cell quiescence. Nature 502, 637–643.
Li, C.L., and Johnson, G.R. (1994). Stem cell factor enhances the survival but not the self-renewal of murine hematopoietic long-term repopulating cells. Blood 84, 408–414.

Li, T., Huang, J., Jiang, Y., Zeng, Y., He, F., Zhang, M.Q., Han, Z., and Zhang, X. (2009). Multi-stage analysis of gene expression and transcription regulation in C57/B6 mouse liver development. Genomics 93, 235–242.

Li, Z., Lan, Y., He, W., Chen, D., Wang, J., Zhou, F., Wang, Y., Sun, H., Chen, X., Xu, C., et al. (2012). Mouse embryonic head as a site for hematopoietic stem cell development. Cell Stem Cell 11, 663–675.

Lin, M.I., Price, E.N., Boatman, S., Hagedorn, E.J., Trompouki, E., Satishchandran, S., Carspecken, C.W., Uong, A., DiBiase, A., Yang, S., et al. (2015). Angiopoietin-like proteins stimulate HSPC development through interaction with notch receptor signaling. eLife 4, e05544.

Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell 161, 1202–1214.

Manesia, J.K., Xu, Z., Broekaert, D., Boon, R., van Vliet, A., Eelen, G., Vanwelden, T., Stegen, S., Van Gastel, N., Pascual-Montano, A., et al. (2015). Highly proliferative primitive fetal liver hematopoietic stem cells are fueled by oxidative metabolic pathways. Stem Cell Res 15, 715–721.

McKinney-Freeman, S., Cahan, P., Li, H., Lacadie, S.A., Huang, H.T., Curran, M., Loewer, S., Naveiras, O., Kathrein, K.L., Konantz, M., et al. (2012). The transcriptional landscape of hematopoietic stem cell ontogeny. Cell Stem Cell 11, 701–714.

Medvinsky, A., and Dzierzak, E. (1996). Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86, 897–906.

Metcalf, D. (2008). Hematopoietic cytokines. Blood 111, 485–491.

Mikkola, H.K.A., and Orkin, S.H. (2006). The journey of developing hematopoietic stem cells. Development 133, 3733–3744.

Mochizuki-Kashio, M., Mishima, Y., Miyagi, S., Negishi, M., Saraya, A., Konuma, T., Shinga, J., Koseki, H., and Iwama, A. (2011). Dependency on the polycomb gene Ezh2 distinguishes fetal from adult hematopoietic stem cells. Blood 118, 6553–6561.

Morrison, S.J., Hemmati, H.D., Wandycz, A.M., and Weissman, I.L. (1995). The purification and characterization of fetal liver hematopoietic stem cells. Proc Natl Acad Sci USA 92, 10302–10306.

Morrison, S.J., and Scadden, D.T. (2014). The bone marrow niche for haematopoietic stem cells. Nature 505, 327–334.

Munugalavadla, V., Dore, L.C., Tan, B.L., Hong, L., Vishnu, M., Weiss, M.J., and Kapur, R. (2005). Repression of c-kit and its downstream substrates by GATA-1 inhibits cell proliferation during erythroid maturation. Mol Cellular Biol 25, 6747–6759.

Orkin, S.H., and Zon, L.I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132, 631–644.

Peng, G., Suo, S., Chen, J., Chen, W., Liu, C., Yu, F., Wang, R., Chen, S., Sun, N., Cui, G., et al. (2016). Spatial transcriptome for the molecular annotation of lineage fates and cell identity in Mid-gastrula mouse embryo. Dev Cell 36, 681–697.

Porayette, P., and Paulson, R.F. (2008). BMP4/Smad5 dependent stress erythropoiesis is required for the expansion of erythroid progenitors during fetal development. Dev Biol 317, 24–35.

Rybtsov, S., Ivanovs, A., Zhao, S., and Medvinsky, A. (2016). Concealed expansion of immature precursors underpins acute burst of adult HSC activity in foetal liver. Development 143, 1284–1289.

Swain, A., Inoue, T., Tan, K.S., Nakanishi, Y., and Sugiyama, D. (2014). Intrinsic and extrinsic regulation of mammalian hematopoiesis in the fetal liver. Histol Histopathol 29, 1077–1082.

Szilvassy, S.J., Meyerrose, T.E., Ragland, P.L., and Grimes, B. (2001). Differential homing and engraftment properties of hematopoietic progenitor cells from murine bone marrow, mobilized peripheral blood, and fetal liver. Blood 98, 2108–2115.

Szpinda, M., Paruszewska-Achtel, M., Woźniak, A., Badura, M., Mila-Kierzenkowska, C., and Wiśniewski, M. (2015). Three-dimensional growth dynamics of the liver in the human fetus. Surg Radiol Anat 37, 439–448.

Takebe, T., Sekine, K., Suzuki, Y., Enomura, M., Tanaka, S., Ueno, Y., Zheng, Y.W., and Taniguchi, H. (2012). Self-organization of human hepatic organoid by recapitulating organogenesis *in vitro*. Transplant Proc 44, 1018–1020.

Tang, Y., Peitzsch, C., Charoudeh, H.N., Cheng, M., Chaves, P., Jacobsen, S.E.W., and Sitnicka, E. (2012). Emergence of NK-cell progenitors and functionally competent NK-cell lineage subsets in the early mouse embryo. Blood 120, 63–75.

Treutlein, B., Brownfield, D.G., Wu, A.R., Neff, N.F., Mantalas, G.L., Espinoza, F.H., Desai, T.J., Krasnow, M.A., and Quake, S.R. (2014). Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature 509, 371–375.

Williams, D.E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C.J., Park, L.S., Martin, U., Mochizukl, D.Y., Boswell, H.S., et al. (1990). Identification of a ligand for the c-kit proto-oncogene. Cell 63, 167–174.

Xie, Y., Li, Y., and Kong, Y. (2014). OPN induces FoxM1 expression and localization through ERK 1/2, AKT, and p38 signaling pathway in HEC-1A cells. Int J Mol Sci 15, 23345–23358.

You, L., Li, L., Zou, J., Yan, K., Belle, J., Nijnik, A., Wang, E., and Yang, X.J. (2016). BRPF1 is essential for development of fetal hematopoietic stem cells. J Clin Investig 126, 3247–3262.

Zayas, J., Spassov, D.S., Nachtman, R.G., and Jurecic, R. (2008). Murine hematopoietic stem cells and multipotent progenitors express truncated intracellular form of c-kit receptor. Stem Cells Dev 17, 343–354.

Zeigler, F.C., Bennett, B.D., Jordan, C.T., Spencer, S.D., Baumhueter, S., Carroll, K.J., Hooley, J., Bauer, K., and Matthews, W. (1994). Cellular and molecular characterization of the role of the flk-2/flt-3 receptor tyrosine kinase in hematopoietic stem cells. Blood 84, 2422–2430.

Zhang, C.C., Kaba, M., Ge, G., Xie, K., Tong, W., Hug, C., and Lodish, H.F. (2006). Angiopoietin-like proteins stimulate *ex vivo* expansion of hematopoietic stem cells. Nat Med 12, 240–245.

Zhang, C.C., and Lodish, H.F. (2004). Insulin-like growth factor 2 expressed in a novel fetal liver cell population is a growth factor for hematopoietic stem cells. Blood 103, 2513–2521.

Zhao, X., Gao, F., Zhang, X., Wang, Y., Xu, L., Liu, K., Zhao, X., Chang, Y., Wei, H., Chen, H., et al. (2016). Improved clinical outcomes of rhG-CSF-mobilized blood and marrow haploidentical transplantation compared to propensity score-matched rhG-CSF-primed peripheral blood stem cell haploidentical transplantation: a multicenter study. Sci China Life Sci 59, 1139–1148.

Zhao, Y., Zhou, J., Liu, D., Dong, F., Cheng, H., Wang, W., Pang, Y., Wang, Y., Mu, X., Ni, Y., et al. (2015). ATF4 plays a pivotal role in the development of functional hematopoietic stem cells in mouse fetal liver. Blood 126, 2383–2391.

Zheng, J., Umikawa, M., Cui, C., Li, J., Chen, X., Zhang, C., Huynh, H.D., Hyunh, H., Kang, X., Silvany, R., et al. (2012). Inhibitory receptors bind ANGPTLs and support blood stem cells and leukaemia development. Nature 485, 656–660.

Zhou, B.O., Yu, H., Yue, R., Zhao, Z., Rios, J.J., Naveiras, O., and Morrison, S.J. (2017). Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF. Nat Cell Biol 19, 891–903.