Correspondence on Letter regarding “Liver Sinusoidal Endothelial Cell: An Important Yet Often Overlooked Player in the Liver Fibrosis”

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Running title: Response to letter on LSEC’ role in liver fibrosis

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Yasuko Iwakiri recently shared the professional views regarding our review on the role of liver sinusoidal endothelial cells (LSECs) in the process of liver fibrosis. The author provided a comprehensive understanding of the structural changes of LSECs including fenestrae and capillarization, nitric oxide (NO) dependent LSECs-HSCs communication, the scavenging capacity of LSECs and the heterogeneity of LSECs.

In liver, a fusion of nutrient-rich blood from the portal vein’s terminal branches with oxygen-enriched blood from the hepatic artery occurs. Then, the mixed blood travels through an intricate network of sinusoidal vessels, and subsequently entering the central venules and hepatic vein. These sinusoids are enveloped by LSECs, whose distinctive position dictates its function. Based on the phagocytic capability of Kupffer cells, most past studies held the belief that clearance within the liver was primarily orchestrated by these cells [1]. Currently, an increasing number of studies have revealed that despite LSECs normally lacking phagocytic capability, they could also transport lipoprotein via fenestrae as well as clear wastes and pathogens in blood via scavenger receptors-controlled endocytosis [2]. Given that the fenestrae of LSECs not only serve as a hallmark of healthy LSECs but also fulfill crucial functions, the number of the related researches were increasing but still need lack of study. For example, apart from scanning electron microscopy, there were no specific molecular markers that could represent fenestrae. Plasmalemma vesicle-associated protein (PLVAP) is a dimeric protein constituent of the diaphragm of diaphragmed-fenestrae, whose deficiency results in the lack of fenestrations. During liver fibrosis, the number of fenestrations were supposed to decrease. However, the PLVAP expression of LSECs
was significant upregulated in all zones in cirrhotic livers [3], indicating that PLVAP might affect other LSEC functions except fenestrae. Therefore, it was in urgent need to find a maker of LSEC fenestrae. Furthermore, except for cytoskeleton remodeling, the recent research reported that intracellular protein disulfide isomerase A1 (PDIA1) showed regulation in the dynamics of LSEC fenestrae [4]. The reduction of fenestrae due to PDIA1 inhibition did not accompany significant cytoskeletal rearrangement. This complements our research finding on the dynamic reshaping of the cytoskeleton [5].

At present, the majority of the researchers that focused on LSECs, distinguished LSECs by the traditional and canonical endothelial cells (ECs) markers such as CD31, VEGFR2, LYVE1. However, none of these markers are liver-specific. On the one hand, LSECs and ECs shared same markers such as CD31 and VEGFR2. On the other hand, the recognized LSECs marker, LYVE1 also showed abundant expression in lymphatics and lung tissues. Therefore, it was in urgent need to find LSECs markers that could distinguish LSECs from ECs and specifically expressed in liver region. LSECs-specific Cre mouse model will help unraveling the intricate biology of LSECs in both health and disease hold immense promise for the development of targeted therapies. Interestingly, a current study has identified oncoprotein-induced transcript 3 (Oit3) as a promising hallmark gene for targeting LSECs through single-cell RNA sequencing (scRNA-seq) [6]. They also established Oit3-CreERT2-tdTomato mice and found tdTomato only showed in liver rather than other organs. Furthermore, tdTomato-labeled cells were not only distinguishable from other types of liver cells but also exhibited CD31 positivity in 98.76% of cases and co-localized with Lyve1, indicating that Oit3 was worthy for further
investigations and applications.

Detecting differentially expressed genes in primary LSECs through quantitative real-time PCR or RNA-seq was a pivotal method for studying the mechanisms underlying LSEC functions and elucidating the pharmacological mechanisms of drugs targeting LSECs. However, the attainment of pure isolation of LSECs was challenging due to the possibility of contamination with other cell types, thereby potentially influencing the overall interpretation of results. With the development of the advancements in scRNA-seq, this problem was probably solved. As reported, human ECs were identified as CD45$^{\text{neg}}$CD31$^{\text{CD38}^+}$neg cells, with sinusoids characterized phenotypically as CD45$^{\text{neg}}$CD31$^{\text{CD38}^+}$neg CD14CD34flow, and large vessels as CD45$^{\text{neg}}$CD31$^{\text{CD38}^+}$neg CD14CD34$^{\text{high}}$CD9 [7]. Furthermore, considerable attention has been directed towards understanding the importance of LSEC heterogeneity in both healthy and injured conditions. As Yasuko Iwakiri mentioned, there were two LSECs population identified as CD34+PLVAP$^+$VWA1+ and CD34+PLVAP$^+$ACKR1+ based on the scRNA-seq from normal and cirrhotic patients. Another study identified two subpopulations of LSECs including Egr1hi LSECs and Ly6ahi LSECs emerged in the early stage of non-alcoholic steatohepatitis (NASH) progression and experienced dramatic proliferation with the aggressive of NASH [8]. Notably, a cluster of C-Kit-positive LSECs was proved to restore mitophagy and alleviate NASH development. Thus, demonstrating the different functions of subpopulations of LSECs might be benefit to reveal the complex roles of LSECs in liver fibrosis.

Given the fact that there is no FDA approved anti-fibrotic drugs for liver fibrosis,
this remains a field ripe with exploration potential. Blood-tonifying and blood-activating herbs that belongs to traditional Chinese medicine have shown therapeutic effects on angiogenesis-related conditions such as cardiovascular diseases and ischemia-reperfusion injury in clinical practice [9, 10]. Li et al. extensively detailed Chinese herbs renowned for their ability to inhibit pathological angiogenesis, aiming to alleviate liver fibrosis [11]. In our review, we also reported that Fuzhenghuayu and Xuefuzhuyu significantly inhibited the dedifferentiation of LSECs, suggesting further investigation into Chinese herbs and related components regulating LSECs and their regulatory mechanisms.

Overall, we found these professional comments quite inspiring for the future development about the researches that aimed at unraveling the intricate biology of LSECs in both health and disease hold immense promise for the development of targeted therapies. Comprehensive understanding of LSEC heterogeneity, molecular signaling pathways, and their dynamic interplay within the liver microenvironment will undoubtedly pave the way for more effective treatments for liver fibrosis and its associated complications.

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References