REVIEW

Development and aging of resident endothelial stem cells in pre-existing blood vessels

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Organ-specific somatic stem cells play an important role in supporting tissue turnover and facilitating regeneration on injury. Hematopoietic stem cells are one of the most established organ-specific somatic cells that have been frequently used for transplantation therapy. Recently, there has been a growing interest in other organ-specific somatic cells, including vascular endothelial stem cells (VESCs). We have previously reported on the use of CD157 and CD200 as markers to isolate VESCs from adult mouse organs, particularly the liver. In this review, we aimed to summarize, based on our previous research, how CD157$^+$CD200$^+$ VESCs in the liver develop from the fetal stage to postnatal life, what transcriptional regulatory mechanisms govern them, and how VESCs change with aging.

HIGHLIGHTS

- Fetal CD157$^-$CD200$^+$ endothelial cells (ECs) are highly proliferative and give rise to adult CD157$^+$CD200$^+$ ECs.
- Aging reduces the number of CD157$^+$CD200$^+$ ECs and impairs their colony-forming capacity and reconstitution ability upon transplantation.
- Enhanced inflammatory responses contribute to the aging of CD157$^+$CD200$^+$ ECs.

INTRODUCTION

The integrity of each organ is maintained by organ-specific somatic stem cells [1–11]. However, such cells have not been isolated from all organs. This partly arises from the difficulty of defining a cell fraction, considered stem cells, as actual stem cells, even if such a fraction is discovered. In the field of stem cell biology, the term "stem cell" must first meet the criteria of maintaining an undifferentiated state and having the ability to produce identical undifferentiated cells through self-renewal [12–14]. This includes symmetric cell division, where both daughter cells remain as stem cells, as well as asymmetric cell division, in which one daughter cell remains a stem cell whereas the other differentiates [15,16].

Another essential criterion for defining stem cells is their differentiation potential. Stem cells must occupy the apex of a cellular hierarchy, continuously generating various organ-specific cells [17]. To meet these two criteria, experimental techniques must demonstrate that a single transplanted cell, defined as a stem cell, persists in the recipient organ over the long term, ideally increasing in number through self-renewal [18–20]. Moreover, as the ultimate condition, it must be shown that these candidate stem cells derived from the first transplantation can function as the apex of the hierarchy when transplanted into a secondary recipient [21].

Of the cell fractions identified as stem cells in various organs, only hematopoietic stem cells (HSCs) have met the ultimate conditions described above and have long been used in transplantation therapy [22,23]. HSCs have the advantage of being easily collected from their niche, the bone marrow, making them highly accessible as material for basic research, which has significantly advanced the study of these cells [24,25].

Other types of stem cells, such as neural, intestinal epithelial, and epidermal stem cells, have been studied over the past two to three decades [1,5,11]. In contrast, however, their specific localization in niches, such as the hippocampus, crypts, and bulge regions, respectively, has made it challenging for most researchers to readily dissociate organs, prepare single-cell suspensions, and analyze cellular functions. Recently, the establishment of research techniques, such as organoid culture, has allowed for greater progress in the study of intestinal epithelial stem cells compared with other stem cell niches [26,27]. Nonetheless, these cells have not yet become a widely utilized tool of research.

Our research group has long focused on isolating vascular endothelial stem cells (VESCs) that exist within pre-existing blood vessels

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and that do not originate from the bone marrow. This focus is based on studies we have conducted treating angiogenesis induced by HSCs [28] and, conversely, the formation of HSC niches by blood vessels as interorgan interactions [29].

In addition to these studies, we have reported, for example, that HSCs under specific conditions transform into pericyte-like cells, contributing to the transient stabilization of immature blood vessels [30]. We have also demonstrated that blood cells act as migratory factors, triggering the sprouting of new blood vessels from pre-existing ones within tumors [31]. Moreover, we have elucidated the mechanism behind the parallel alignment of arteries and veins, showing that neutrophils induce this parallelism through the apelin/apelin receptor signaling pathway [32].

Based on the finding of such close relationships between blood cells and vascular endothelial cells (ECs), we hypothesized that, just as HSCs exhibit a hierarchical differentiation system, a hierarchy might also exist within pre-existing blood vessels with VESCs at their apex. Thus, we have been investigating whether VESCs exist within pre-existing blood vessels.

Initially, we discovered heterogeneity in ECs within pre-existing blood vessels, particularly in their proliferative capacity. Highly proliferative ECs were predominantly observed among side population (SP) cells, which exhibit high expression of ABC transporters. These cells rapidly efflux the Hoechst dye, rendering them unstained by it. Conversely, main population (MP) cells that retained Hoechst dye rarely included ECs with high proliferative capacity. Endothelial SP cells were found in organs other than the bone marrow and demonstrated high proliferative activity [33–36].

When endothelial SP cells isolated from skeletal muscle were transplanted into muscle showing ischemia by femoral artery ligation in mice, they contributed to the ECs of all blood vessels across several millimeters, forming long-lasting vessels [35]. Similarly, when endothelial SP cells derived from the liver were transplanted into a liver injury model in mice, they induced continuous vascular formation and contributed to ECs of the portal vein, hepatic vein, and sinusoidal vessels [37].

Although SP cells differentiated into MP cells, reisolating SP and MP cells from ECs derived from SP cells and serially transplanting them showed that only SP cells retained the ability to reconstruct blood vessels [35,37]. This finding indicated that SP cells occupy the top of the differentiation hierarchy. However, the transition from SP to MP cells might simply reflect a change in EC status from drug-efflux capability to noncapability.

To address the phenotypical hierarchy of ECs, we analyzed differentially expressed genes between SP and MP cells. Through this analysis, we found that vascular ECs expressing both CD157 and CD200 —cell surface glycoproteins—represent the apex of the hierarchy and are the true VESCs [37–39]. Here, we discussed our previous findings regarding the origin of $CD157^+CD200^+$ ECs and the molecular mechanism regulating their development. We also discussed the decline in functionality associated with aging.

OVERVIEW OF EARLY EC DEVELOPMENT

To understand how $CD157^+CD200^+$ ECs develop in the liver, it is essential to look at the earliest stage of EC development. Hemangioblasts ($Etv2^+$ cells), which are the common precursors to both endothelial and hematopoietic lineages, were first identified in mice at embryonic day (E) 7.0–7.5 [40]. Single-cell RNA sequencing (scRNA-seq) from the endoderm, associated mesoderm, and liver buds showed that hematopoietic cells were derived from hemangioblasts after the activation of transcription factors (TFs) *Runx1* and *Gata1* around E7.5 [41–43]. In the comparison, ECs follow an independent differentiation trajectory characterized by the upregulation of genes, including *Cdh5, Pecam1*, and *Cd34*, around E8.75. ECs also expressed TFs associated with angiogenesis and mechanosensitive ion channels, such as *Klf2/4* and *Piezo1/2*, respectively [43]. Other scRNA-seq data from Sox17-expressing lineages at E9.0–9.5 revealed at least 3 transcriptionally distinct EC subtypes. EC1 (*Clec1b* and *Nid2*) was enriched for mitosis-associated TFs, such as *Ccnb2* and *Tuba1c*, and has the potential to transition into hemogenic endothelium (*Cd44* and *Igfbp5*). EC2 was a nonhemogenic endothelium, predominantly in the S phase of the cell cycle, enriched in the expression of *Hist1h1b* and *Hist1h2ae*. EC3 (*Hoxa9* and *Hoxb7*) is believed to be derived from the yolk sac as it expressed *Hspd1*, a gene necessary for yolk-sac erythropoiesis. This population was described as a bipotent progenitor that can give rise to either EC2 or erythroid cells (*Gypa* and *Alas2*) [44] (Figure 1).

The liver vasculature begins to develop within the liver bud at E8.5–E9.0 through the infiltration of progenitors from the adjacent vasculature [45]. Histologically, LSECs are first observed around E9.5–E10.5, but transcriptional divergence from ECs toward LSECs can be detected as early as E8.75. This transition is characterized by the upregulation of sinusoidal-specific genes, including *Oit3, Lyve1*, and *Stab2* [43,46]. Multiple sources may contribute to LSEC development, including the vitelline and posterior cardinal veins, sinus venous-derived $NFATC1^+/NPR3^-$ progenitor cells, and $CSF1R^+$ erythroid–myeloid progenitors [47–49]. Along with LSECs, portal and hepatic veins also expand around E11.0, with hepatic veins arising from vitelline veins and posterior cardinal veins, although portal veins originate from the vitelline veins [45,50].

$CD157^-CD200^+$ ECs as a Stem Cell-containing Fraction During Late-Stage Fetal Liver Development

The stem-like properties of mid-fetal liver macrovascular ECs and LSECs seem to be associated with CD34 expression. Liver EC scRNA-seq analysis indicated that both macrovascular ECs and LSECs, including proliferating ECs, expressed CD34 until approximately E12 [51]. $SCL^-PLAP^+VeCad^+CD45^-$ cells from E12 fetal liver were also reported to have long-term endothelial (LTR-EC) reconstitution activity on transplantation into neonatal mice. This population expressed CD31, Tie2, Flk1, and CD34, lacked the expression of the hematopoietic receptor Mac-1, and was mostly composed of $Lyve1^+$ LSECs [52].

At E14, almost 90% of $VeCad^+CD45^-$ cells were $Lyve1^+$LSECs, but the LTR-EC activity was greatly reduced compared with E12 [52]. Transcriptionally, at E14, most of the LSECs had lost CD34 expression, although most macrovascular ECs remained $CD34^+$ and began expressing CD200 [51,53]. $CD200^+$ ECs gradually expanded in number throughout late fetal development and, by E18, were widely distributed across portal, hepatic, and vitelline/umbilical veins. After birth, the vitelline/umbilical vein regressed, limiting CD200 expression to the portal vein, hepatic vein, and the newly established hepatic artery. In adulthood, $CD200^+$ ECs dominated these vascular regions as their population expanded [53].

Colony-forming assays showed that both $CD200^+$ and $CD200^-$ ECs possessed high proliferative capacity on embryonic day E18, suggesting the presence of endothelial stem/progenitor cells within both

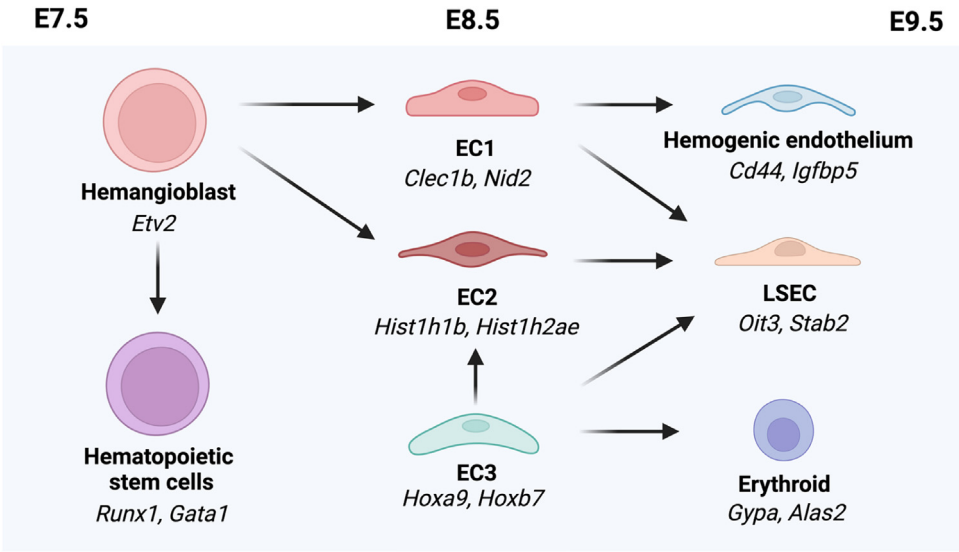

Figure 1 During early embryonic development, hemangioblasts give rise to two distinct trajectories: hematopoietic and endothelial cell lineages. Although all endothelial cells (ECs) express common endothelial markers, they can be transcriptionally divided into at least 3 subtypes: EC1 and EC2 are derived from hemangioblasts whereas EC3 is believed to migrate from extraembryonic tissues. Both EC1 and EC3 have the potential to transition into hematopoietic lineages, giving rise to hemogenic endothelium and erythroid cells, respectively. Liver sinusoidal endothelial cells (LSECs) are thought to emerge from a common EC progenitor. Created with BioRender.com.

populations. $CD200^+$ ECs had a significantly higher colony-forming capacity, with endothelial colony-forming cell (ECFC) frequencies detected at 1 in 8 cells for $CD200^+$ ECs, but only 1 in 62 cells for $CD200^-$ ECs. After birth, $CD200^-$ ECs significantly lost their proliferative potential, with an ECFC frequency of 1 in 748 by postnatal day 7 (P7) and an inability to form colonies. In contrast, $CD200^+$ ECs exhibited a gradual decline in proliferation following the emergence of two subpopulations, $CD157^+CD200^+$ and $CD157^-CD200^+$, marked by the onset of CD157 expression. By P21, only 1 in 133 $CD157^-CD200^+$ cells retained ECFC characteristics, forming fewer and smaller colonies [53] (Figure 2).

Transplantation experiments demonstrated that $CD200^+$ and $CD200^-$ ECs from E18 contributed to long-term EC reconstitution after monocrotaline (MCT)-induced liver injury, resulting in the generation of cell populations, i.e., $CD157^-CD200^-$, $CD157^-CD200^+$, and $CD157^+CD200^+$ ECs from $CD200^+$ and $CD200^-$ ECs. However, $CD200^+$ ECs have been found to regenerate the vascular system better with a more extensive vascular network and a higher number of ECs. Additionally, $CD200^+$ ECs have a higher capacity to produce $CD157^+CD200^+$ cells than $CD200^-$ ECs, providing evidence that the stem cell population is primarily located in the $CD157^-CD200^+$ EC fraction during late-stage fetal development [53]. In the adult liver, $CD157^-CD200^+$ ECs only gave rise to $CD157^-CD200^+$ ECs and $CD157^-CD200^-$ ECs, indicating a transition of $CD157^-CD200^+$ ECs to a progenitor state, whereas transplanted adult $CD157^-CD200^-$ ECs only generated very few $CD157^-CD200^-$ ECs displaying characteristics of mature ECs [37].

On a side note, $CD157^-CD200^+$ ECs from the prenatal period also expressed embryonic and neonatal endothelial progenitor markers identified in previous studies, such as *Peg3/PW1* and *Prom1/Cd133* [53]. $PW1^+$ECs were strongly expressed in the yolk sac and embryonic vasculature, remaining abundant until 1 week after birth before disappearing in adult mouse ECs. This population was highly proliferative, capable of forming colonies in vitro and generating new vessels in vivo [54]. Similarly, $Prom1^+$ECs also gave rise to EC colonies in vitro and participated in vascular repair and remodeling after vascular injury. Although its expression during embryonic development was unknown, *Prom1* was highly expressed in pulmonary macrovascular ECs at 2 weeks of age in mice, after which its expression was greatly reduced in adulthood [55].

Emergence of $CD157^+CD200^+$ ECs in the Liver

Although CD157 mRNA expression was induced in $CD200^+$ ECs by E18, the emergence of $CD157^+CD200^+$ ECs is prominent during the first week of life, predominantly in the portal vein, and then expands to the hepatic vein. This population increases continuously and peaks at 6 months of age before plateauing during adulthood, representing $3.9\% \pm 0.7\%$ of total ECs in the liver. $CD157^+CD200^+$ ECs had a higher capacity for forming larger and more abundant EC colonies than $CD157^-CD200^+$ ECs in an in vitro culture setting. Additionally, the frequency of ECFCs in the $CD157^+CD200^+$ population also increased with age, from 1 in 40 cells at P7 to 1 in 12 cells at P21 [53]. Single adult $CD157^+CD200^+$ ECs were capable of reconstituting functional blood vessels, including the portal vein, hepatic vein, and sinusoids in the MCT-induced liver injury model. In addition, these cells generated all 3 EC fractions ($CD157^+CD200^+$ ECs, $CD157^-CD200^+$ ECs, and $CD157^-CD200^-$ ECs), placing them at the top of the EC hierarchy [37].

The exact mechanisms by which $CD200^+$ ECs become $CD157^+CD200^+$ ECs are not yet fully elucidated. Nonetheless, numerous TFs, such as *Atf3, Bhlhe40, Egr1, Egr2, Elf3*, and *Klf4*, have been proposed as players in this phenomenon. Transduction of these TFs in MS1 cells has been shown to cause an upregulation of CD157

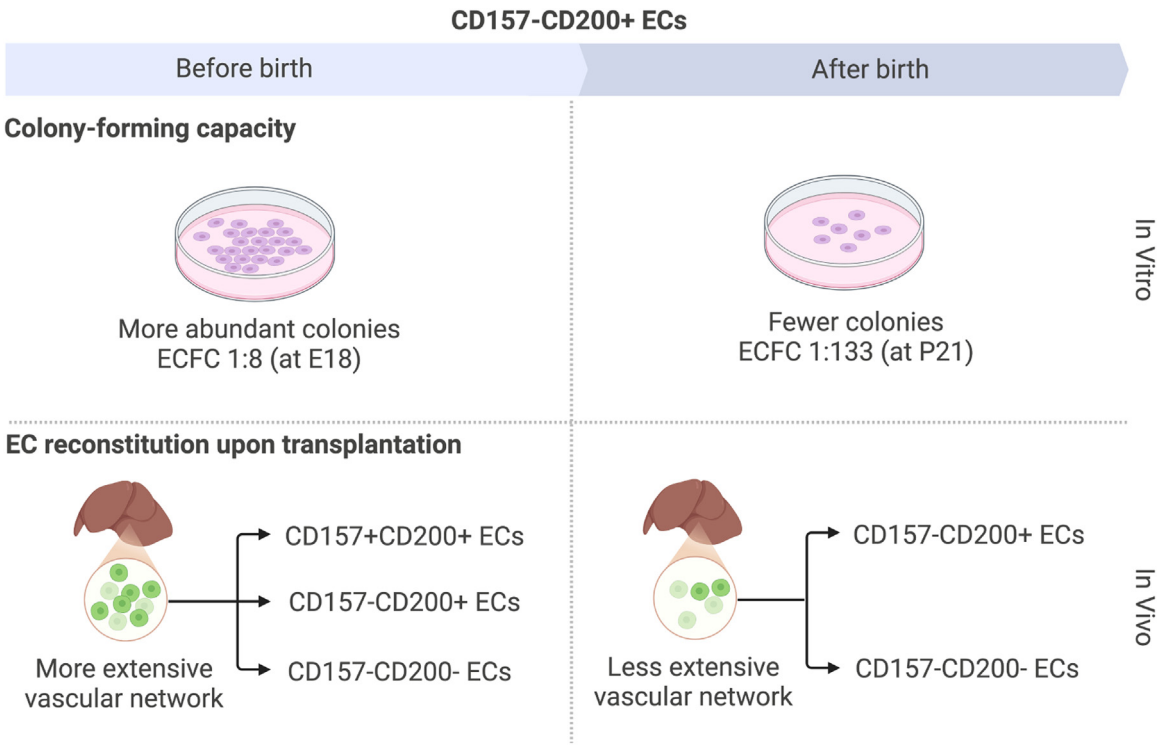

Figure 2 Comparison of the proliferative capacity of CD157^{-}CD200^{+} endothelial cells (ECs) before and after birth shows that such cells at embryonic day 18 (E18) contain a higher number of endothelial colony-forming cells (ECFCs) than those from postnatal day 21 (P21). When transplanted into the host liver, CD157^{-}CD200^{+} ECs from E18 generate a more extensive vascular network and give rise to all three EC fractions. Created with BioRender.com

and ABC transporter expression along with increased proliferation and tube formation. One of these TFs, *Klf4*, has been shown to bind directly to the promoters of CD157 and ABCG2. Interestingly, *Klf4* was highly expressed in CD157^{+}CD200^{+} ECs from E18 until adulthood, emphasizing its importance in the development and maintenance of endothelial stem cells [56].

Beyond the liver, CD157^{+}CD200^{+} ECs have been identified in multiple organs, including the lungs, heart, brain, limb muscles, skin, retina, adipose tissues, pancreas, and placenta [34,37–39]. Based on the scRNA-seq from various organs, this VESC population was primarily located in the veins and postcapillary venules, which may serve as a VESC niche. CD157^{+}CD200^{+} ECs strongly expressed Bmx, and a 12-month observation using Bmx (PAC)-CreERT2/Flox-CAT-enhanced green fluorescence protein (EGFP) mice revealed that EGFP-positive VESCs in the portal vein can generate all liver blood vessels, including sinusoids, under steady-state conditions [37]. In addition, previous lineage-tracing studies also demonstrated that venous ECs serve as the origin of angiogenesis in mice [57]. However, the specific cellular components of the VESC niche and the regulatory signaling mechanism governing VESC maintenance and differentiation remain unknown [58,59].

Relationship With Previously Reported Endothelial Stem/Progenitor Cells Population

CD157^{+}CD200^{+} ECs may also be part of a stem or progenitor cell population described in previous studies, as this EC fraction exhibited a higher expression of *Procr* and *Il33* compared with another EC fraction [53]. Procr^{+}ECs have been identified as bipotent VESCs capable of giving rise to both ECs and pericytes in multiple tissues, including the mammary gland, skin, and retina [60]. Isolated Procr^{+}ECs from the mammary gland demonstrated significantly higher in vitro colony-forming potential—approximately 20 times greater than Procr^{-}ECs. In contrast, *Il33* has been described as one of the genes expressed by endovascular progenitors (EVPs CD31^{low}/VEGFR2^{low}) found in the aorta, lung, placenta, and tumors. Compared with other EC populations, termed transit amplifying ECs (CD31^{int}/VEGFR2^{low}) and definitive differentiated ECs (CD31^{hi}/VEGFR2^{hi}), only EVP cells have been found to possess self-renewal capacity [61].

Recently, Lin et al. [62] proposed *Abcg2*—one of the genes encoding ABC transporter and enriched in SP cells—as a marker for clonal repopulating ECs. Abcg2^{+}ECs from mice and humans exhibited outstanding vasculogenic potential as demonstrated by serial transplantation. Additionally, lineage tracing using Abcg2CreErt2; ROSA TdTomato (Abcg2TT) mice showed that TdTomato^{+}Abcg2^{+}ECs contributed to the generation of arterial, venous, and capillary ECs under steady-state conditions and after myocardial infarction. Both CD157^{+}CD200^{+} ECs and Abcg2^{+}ECs shared the expression of *Myc* and were enriched in genes related to blood vessel development, extracellular matrix adhesion, and cell-cell adhesion. Immunostaining of the heart, muscle, and lung of Abcg2TT mice revealed that *Abcg2* and *CD157* were rarely coexpressed [63]. However, in the liver, *Abcg2* was expressed in both CD157^{+}CD200^{+} and CD157^{-}CD200^{+} EC populations but was absent in the CD157^{-}CD200^{-} mature EC

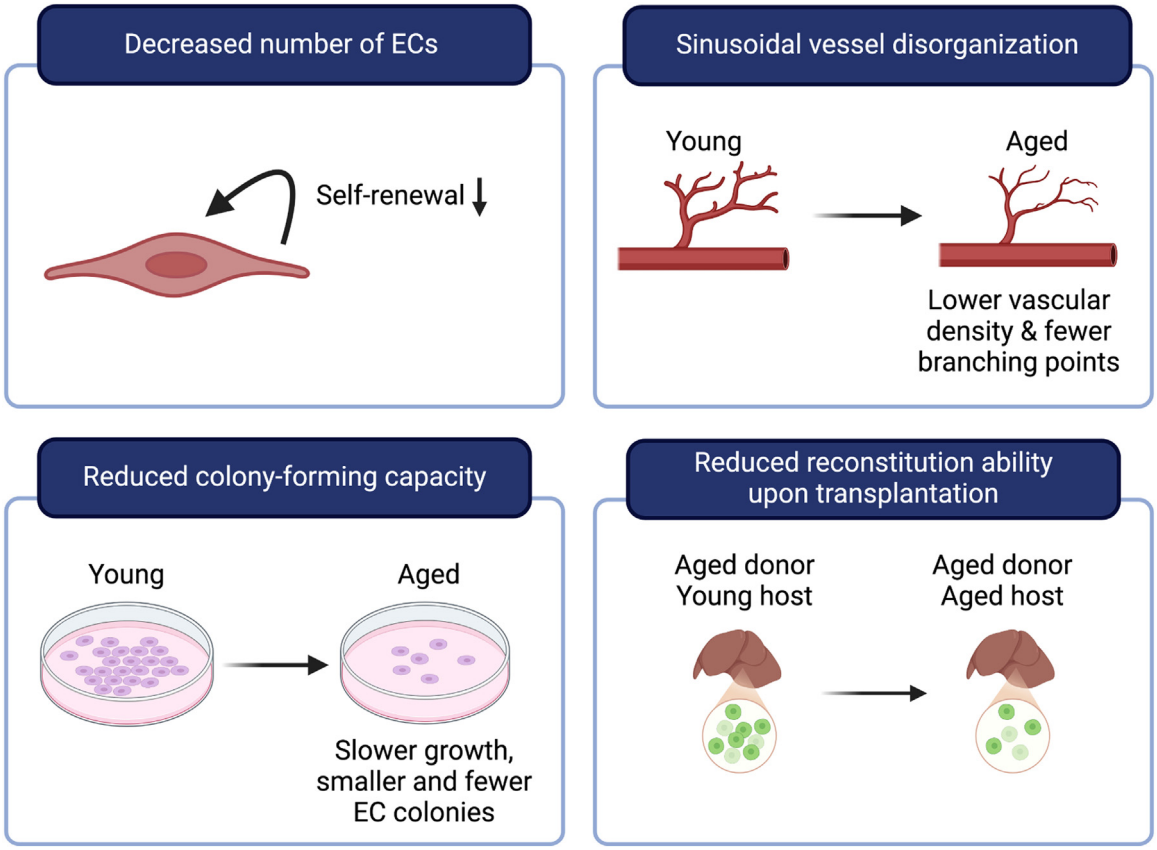

Figure 3 CD157$^+$CD200$^+$ EC functions are altered in aging. Some of these changes include a decreased number of ECs, sinusoidal vessel disorganization, and reduced colony-forming capacity and reconstitution ability of CD157$^+$CD200$^+$ ECs on transplantation. Created with BioRender.com.

population, suggesting that *Abcg2* and *CD157* marked 2 relatively distinct VESC populations [53].

Another study also identified a Lin$^-$CD31$^+$CD105$^+$Sca1$^+$ CD117 (c-kit)$^+$ endothelial progenitor population in the mouse lung vasculature capable of generating functional blood vessels in vivo [64]. Although liver CD157$^+$CD200$^+$ ECs also highly expressed *CD105* and *Sca1, CD117* expression was not specific to VESCs as it was also expressed by pericentral LSECs and served as a sinusoidal zonation marker [53,65,66].

Change of CD157$^+$CD200$^+$ ECs in Aged Microenvironment and Angiogenesis Impairment

Aging is a natural biological process that affects all living organisms, leading to a decline in organ function down to the cellular level. One of the most evident age-related changes in the endothelium is a decline in EC numbers across various mouse organs, as shown in the Tabula Muris Senis dataset [67]. Specifically, in the liver, the total EC population, including CD157$^+$200$^+$ ECs, underwent a significant decrease in 27- to 28-month-old mice [68]. A decline in stem cell numbers is also observed in other adult stem cell types, such as muscle and neural stem cells [69,70]. The decrease in VESC number was accompanied by structural disorganization of liver sinusoidal vessels, including lower vascular density and a reduced number of branching points—signs of impaired angiogenesis [68].

Aging also impairs the regenerative capacity of VESCs. Isolation and in vitro studies showed that CD157$^+$200$^+$ ECs from aged liver and lung have reduced colony-forming capacity, as shown by their slower growth and fewer colonies of a smaller size compared with CD157$^+$200$^+$ ECs from young mice. In in vivo studies, stem cells harvested from aged donor mice generally exhibit much lower self-capacity when transplanted into young recipients compared with stem cells from young donor mice [71]. Interestingly, in vivo experiments showed that upon liver injury, simulated by the intraperitoneal administration of MCT into young mice, transplanted aged CD157$^+$200$^+$ ECs proliferated and integrated into the recipient's liver with an efficiency comparable with that of their young counterparts [68]. This finding suggested that the proliferative capacity of CD157$^+$200$^+$ ECs in vivo is predominantly influenced by the age of the host environment. Similar observations have been reported in transplantation experiments of muscle stem cells where young systemic and microenvironmental factors rejuvenated aged stem cells to restore their proliferative and differentiation capacities [72]. In line with this, aged CD157$^+$200$^+$ ECs reconstitute a less extensive vascular area on transplantation into aged mice compared with young CD157$^+$200$^+$ ECs, confirming that the functionality of these stem cells declined with age [68]. These results emphasized the importance

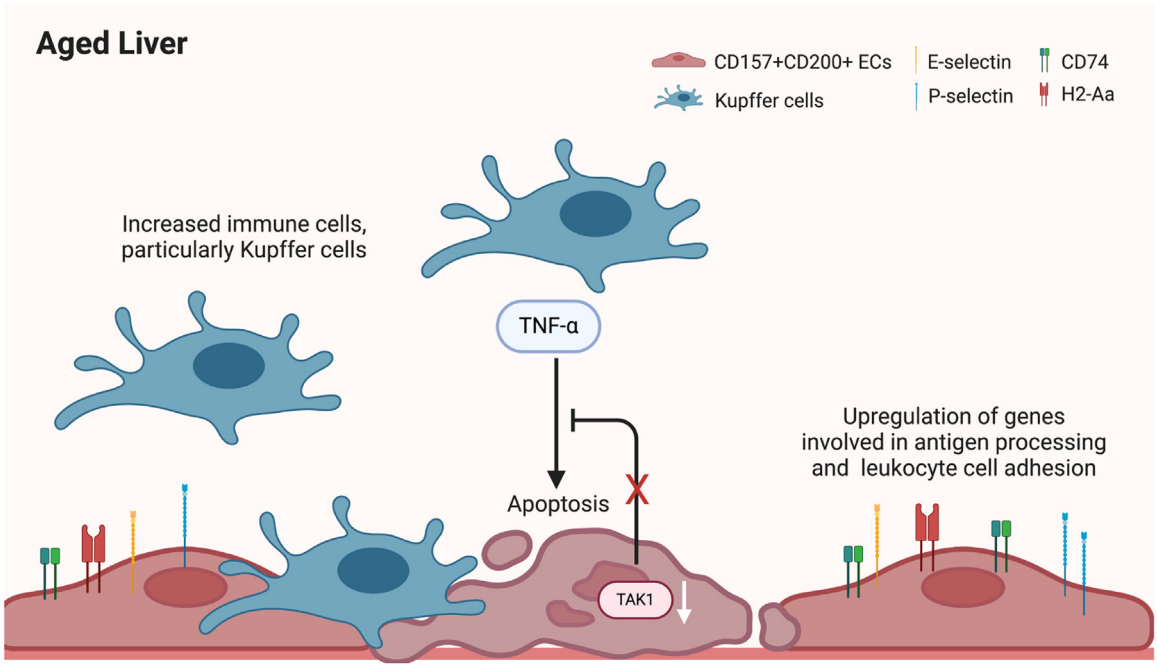

Figure 4 Elevated systemic and local inflammation significantly contributes to the aging of CD157⁺CD200⁺ ECs. Aged ECs show increased expression of genes involved in antigen processing and presentation, leukocyte cell-cell adhesion, and apoptosis regulation. Higher immune cell infiltration, particularly Kupffer cells, releases the proinflammatory factor tumor necrosis factor-α (TNF-α), promoting apoptosis. Additionally, reduced transforming growth factor β-activated kinase 1 (TAK1) expression in aged ECs increases their susceptibility to apoptosis. Created with BioRender.com.

of both systemic and local microenvironmental signals in reprogramming the regenerative capacity of VESCs during aging (Figure 3).

Molecular Mechanism of CD157⁺CD200⁺ EC Aging

One of the major contributors to an age-related decline in proliferation and angiogenesis in ECs, including VESCs, is elevated inflammatory responses in aged systemic and local microenvironments. The scRNA-seq obtained from the Tabula Muris Senis project showed enhanced immune cells and an immune-related gene signature in aged mouse organs [67]. Immune cells were further characterized using immunohistochemical and in situ RNA staining in aged liver tissue, revealing elevated numbers of *CD45*⁺ immune cells and *Clec4f*⁺*Il1b*⁺ Kupffer cells suggestive of a proinflammatory state [68]. Kupffer cells were shown to secrete tumor necrosis factor (TNF)-α in inflammatory conditions, which induces TNF receptor 1 (TNFR1)-mediated apoptosis in ECs [73]. The dysregulation of the proinflammatory cytokine, TNF-α, was also observed systemically, as indicated by an increased plasma level and local release in various organs and tissues, including the heart, carotid arteries, and aortic wall [74–77]. It has been reported that transforming growth factor β-activated kinase 1 (TAK1) played a critical protective role in preventing TNF-α-TNFR1-induced EC apoptosis [78]. Notably, aged liver ECs exhibited reduced expression of TAK1 compared with their younger counterparts, suggesting that TAK1 may also regulate apoptosis in VESCs during aging [68] (Figure 4).

Gene ontology biological processes in aged liver ECs indicated significant enrichment in pathways such as antigen processing and the presentation of exogenous peptide antigen via major histocompatibility complex (MHC) class II, leukocyte cell-cell adhesion, and positive regulation of the apoptotic process. In addition, the expression of genes involved in these pathways, such as *Cd74, H2-Aa, Sele,* and *Selp,* was much higher in CD157⁺200⁺ ECs relative to other EC fractions [68]. Specifically, transcriptional changes in ECs between 18 and 30 months were progressive rather than dichotomous, as shown by scRNA-seq studies of heart and aorta ECs [79]. The slow build-up of inflammatory signals highlights the nonstatic nature of the aging process in the vascular endothelium.

CONCLUSION AND FUTURE DIRECTIONS

The identification of CD157 and CD200 as markers of VESCs, particularly in the liver, has further solidified the existence of a hierarchical lineage of ECs. We have demonstrated that adult CD157⁺CD200⁺ ECs emerge from CD157⁻CD200⁺ ECs during late fetal development. Although we have uncovered several clues regarding the development of CD157⁺CD200⁺ ECs, additional investigation is required to elucidate the molecular mechanism fully behind the induction of VESCs and its niche to utilize this population for effective angiogenesis therapy.

Aging, in comparison, has a powerful effect on diminishing the proper function of CD157⁺CD200⁺ ECs. Aging-associated inflammation, marked by the increased infiltration of immune cells and enhanced levels of inflammation-related genes, disrupts vascular homeostasis, including angiogenesis. However, further research regarding alterations in intrinsic age-related cell and stem cell niches is still needed because a combination of these factors is essential for regulating the deterioration of the stem cell population.

Conflict of Interest Disclosure

The authors have no conflicts of interest to disclose.

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REFERENCES

1. Obernier K, Alvarez-Buylla A. Neural stem cells: origin, heterogeneity and regulation in the adult mammalian brain. Development 2019;146: dev156059.
2. Relaix F, Bencze M, Borok MJ, et al. Perspectives on skeletal muscle stem cells. Nat Commun 2021;12:692.
3. Mehanna RA, Essawy MM, Barkat MA, et al. Cardiac stem cells: current knowledge and future prospects. World J Stem Cells 2022;14:1–40.
4. Pinho S, Frenette PS. Haematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol 2019;20:303–20.
5. Choi J, Augenlicht LH. Intestinal stem cells: Guardians of homeostasis in health and aging amid environmental challenges. Exp Mol Med 2024;56:495–500.
6. Robertson SYT, Roberts JS, Deng SX. Regulation of limbal epithelial stem cells: Importance of the niche. Int J Mol Sci 2021;22:11975.
7. Bonner-Weir S, Weir GC. New sources of pancreatic β-cells. Nat Biotechnol 2005;23:857–61.
8. Dong L, Li X, Leng W, et al. Adipose stem cells in tissue regeneration and repair: From bench to bedside. Regen Ther 2023;24:547–60.
9. Bruno S, Herrera Sanchez MB, Chiabotto G, et al. Human Liver Stem Cells: A liver-derived mesenchymal stromal cell-like population with pro-regenerative properties. Front Cell Dev Biol 2021;9:644088.
10. Jones-Freeman B, Starkey MR. Bronchioalveolar stem cells in lung repair, regeneration and disease. J Pathol 2020;252:219–26.
11. Tang X, Wang J, Chen J, et al. Epidermal stem cells: Skin surveillance and clinical perspective. J Transl Med 2024;22:779.
12. Goodell MA, Nguyen H, Shroyer N. Somatic stem cell heterogeneity: Diversity in the blood, skin and intestinal stem cell compartments. Nat Rev Mol Cell Biol 2015;16:299–309.
13. Weissman IL. Stem cells: Units of development, units of regeneration, and units in evolution. Cell 2000;100:157–68.
14. Morrison SJ, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. Cell 1997;88:287–98.
15. Chhabra SN, Booth BW. Asymmetric cell division of mammary stem cells. Cell Div 2021;16:5.
16. Fuchs E, Chen T. A matter of life and death: Self-renewal in stem cells. EMBO Rep 2013;14:39–48.
17. Beumer J, Clevers H. Hallmarks of stemness in mammalian tissues. Cell Stem Cell 2024;31:7–24.
18. Majeti R, Park CY, Weissman IL. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. Cell Stem Cell 2007;1:635–45.
19. Yang L, Bryder D, Adolfsson J, et al. Identification of Lin-Sca1+kit+CD34+Flt3- short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. Blood 2005;105:2717–23.
20. Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity 1994;1:661–73.
21. Notta F, Doulatov S, Laurenti E, Poeppl A, Jurisica I, Dick JE. Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. Science 2011;333:218–21.
22. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: Past, present, and future. Stem Cell Res Ther 2019;10:68.
23. Ema H, Morita Y, Suda T. Heterogeneity and hierarchy of hematopoietic stem cells. Exp Hematol 2014;42:74–82.e2.
24. Pereira AL, Galli S. Nombela-Arrieta C. Bone marrow niches for hematopoietic stem cells. Hemasphere 2024;8:e133.
25. Kwon M, Kim BS, Yoon S, Oh SO, Lee D. Hematopoietic stem cells and their niche in bone marrow. Int J Mol Sci 2024;25:6837.
26. Tian C-M, Yang M-F, Xu H-M, et al. Stem cell-derived intestinal organoids: A novel modality for IBD. Cell Death Discov 2023;9:255.
27. Takahashi T, Fujishima K, Kengaku M. Modeling intestinal stem cell function with organoids. Int J Mol Sci 2021;22:10912.
28. Takakura N, Watanabe T, Suenobu S, et al. A role for hematopoietic stem cells in promoting angiogenesis. Cell 2000;102:199–209.
29. Takakura N, Huang XL, Naruse T, et al. Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis. Immunity 1998;9:677–86.
30. Yamada Y, Takakura N. Physiological pathway of differentiation of hematopoietic stem cell population into mural cells. J Exp Med 2006; 203:1055–65.
31. Okamoto R, Ueno M, Yamada Y, et al. Hematopoietic cells regulate the angiogenic switch during tumorigenesis. Blood 2005;105:2757–63.
32. Kidoya H, Naito H, Muramatsu F, et al. APJ regulates parallel alignment of arteries and veins in the skin. Dev Cell 2015;33:247–59.
33. Wakabayashi T, Naito H. Cellular heterogeneity and stem cells of vascular endothelial cells in blood vessel formation and homeostasis: Insights from single-cell RNA sequencing. Front Cell Dev Biol 2023;11:1146399.
34. Iba T, Naito H, Shimizu S, Rahmawati FN, Wakabayashi T, Takakura N. Isolation of tissue-resident endothelial stem cells and their use in regenerative medicine. Inflamm Regen 2019;39:9.
35. Naito H, Kidoya H, Sakimoto S, Wakabayashi T, Takakura N. Identification and characterization of a resident vascular stem/progenitor cell population in preexisting blood vessels. EMBO J 2012;31:842–55.
36. Sainz J, Zen AAH, Caligiuri G, et al. Isolation of "side population" progenitor cells from healthy arteries of adult mice. Arterioscler Thromb Vasc Biol 2006;26:281–6.
37. Wakabayashi T, Naito H, Suehiro J ichi, et al. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Cell Stem Cell 2018;22:384–97.
38. Naito H, Wakabayashi T, Ishida M, et al. Isolation of tissue-resident vascular endothelial stem cells from mouse liver. Nat Protoc 2020;15:1066–81.
39. Wakabayashi T, Naito H, Iba T, Nishida K, Takakura N. Identification of CD157-positive vascular endothelial stem cells in mouse retinal and choroidal vessels: Fluorescence-activated cell sorting analysis. Invest Ophthalmol Vis Sci 2022;63:5.
40. Huber TL, Kouskoff V, Fehling HJ, Palis J, Keller G. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 2004;432:625–30.
41. Lacaud G, Gore L, Kennedy M, et al. Runx1 is essential for hematopoietic commitment at the hemangioblast stage of development in vitro. Blood 2002;100:458–66.
42. Yokomizo T, Takahashi S, Mochizuki N, et al. Characterization of GATA-1+ hemangioblastic cells in the mouse embryo. EMBO J 2007;26:184–96.
43. Lotto J, Drissler S, Cullum R, et al. Single-cell transcriptomics reveals early emergence of liver parenchymal and non-parenchymal cell lineages. Cell 2020;183:702–716.e14.
44. Trinh LT, Osipovich AB, Liu B, et al. Single-cell RNA sequencing of sox17-expressing lineages reveals distinct gene regulatory networks and dynamic developmental trajectories. Stem Cells 2023;41:643–57.

45. Swartley OM, Foley JF, Livingston DP, Cullen JM, Elmore SA. Histology atlas of the developing mouse hepatobiliary hemolymphatic vascular system with emphasis on embryonic days 11.5-18.5 and early postnatal development. Toxicol Pathol 2016;44:705–25.
46. Matsumoto K, Yoshitomi H, Rossant J, Zaret KS. Liver organogenesis promoted by endothelial cells prior to vascular function. Science 2001;294:559–63.
47. Zhang H, Pu W, Tian X, et al. Genetic lineage tracing identifies endocardial origin of liver vasculature. Nat Genet 2016;48:537–43.
48. Plein A, Fantin A, Denti L, Pollard JW. Ruhrberg C. Erythro-myeloid progenitors contribute endothelial cells to blood vessels. Nature 2018;562:223–8.
49. Sugiyama Y, Takabe Y, Nakakura T, Tanaka S, Koike T, Shiojiri N. Sinusoid development and morphogenesis may be stimulated by VEGF-Flk-1 signaling during fetal mouse liver development. Dev Dyn 2010;239:386–97.
50. Shiojiri N, Niwa T, Sugiyama Y, Koike T. Preferential expression of connexin37 and connexin40 in the endothelium of the portal veins during mouse liver development. Cell Tissue Res 2006;324:547–52.
51. Gómez-Salinero JM, Izzo F, Lin Y, et al. Specification of fetal liver endothelial progenitors to functional zonated adult sinusoids requires c-Maf induction. Cell Stem Cell 2022;29:593–609.e7.
52. Cañete A, Comaills V, Prados I, et al. Characterization of a fetal liver cell population endowed with long-term multiorgan endothelial reconstitution potential. Stem Cells 2017;35:507–21.
53. Rahmawati FN, Iba T, Naito H, et al. Single-cell sequencing reveals the existence of fetal vascular endothelial stem cell-like cells in mouse liver. Stem Cell Res Ther 2023;14:1–14.
54. Malinverno M, Corada M, Ferrarini L, et al. Peg3/PW1 is a marker of a subset of vessel associated endothelial progenitors. Stem Cells 2017;35:1328–40.
55. Sekine A, Nishiwaki T, Nishimura R, et al. Prominin-1/CD133 expression as potential tissue-resident vascular endothelial progenitor cells in the pulmonary circulation. Am J Physiol Lung Cell Mol Physiol 2016;310:L1130–42.
56. Konishi H, Rahmawati FN, Okamoto N, et al. Discovery of transcription factors involved in the maintenance of resident vascular endothelial stem cell properties. Mol Cell Biol 2024;44:17–26.
57. Lee HW, Xu Y, He L, et al. Role of venous endothelial cells in developmental and pathologic angiogenesis. Circulation 2021;144:1308–22.
58. Kalucka J, de Rooij LPMH, Goveia J, et al. Single-cell transcriptome atlas of murine endothelial cells. Cell 2020;180:764–779.e20.
59. Goveia J, Rohlenova K, Taverna F, et al. An integrated gene expression landscape profiling approach to identify lung tumor endothelial cell heterogeneity and angiogenic candidates. Cancer Cell 2020;37:21–36.e13.
60. Yu QC, Song W, Wang D, Zeng YA. Identification of blood vascular endothelial stem cells by the expression of protein C receptor. Cell Res 2016;26:1079–98.
61. Patel J, Seppanen EJ, Rodero MP, et al. Functional definition of progenitors versus mature endothelial cells reveals key SoxF-dependent differentiation process. Circulation 2017;135:786–805.
62. Eckenstaler R, Benndorf RA. 3D structure of the transporter ABCG2—What's new? Br J Pharmacol 2020;177:1485–96.
63. Lin Y, Gil CH, Banno K, et al. ABCG2-Expressing clonal repopulating endothelial cells serve to form and maintain blood vessels. Circulation 2024;150:451–65.
64. Fang S, Wei J, Pentinmikko N, Leinonen H, Salven P. Generation of functional blood vessels from a single c-kit+ adult vascular endothelial stem cell. PLoS Biol 2012;10:e10011407.
65. Su T, Yang Y, Lai S, et al. Single-cell Transcriptomics reveals zone-specific alterations of liver sinusoidal endothelial cells in cirrhosis. Cell Mol Gastroenterol Hepatol 2021;11:1139–61.
66. Duan JL, Zhou ZY, Ruan B, et al. Notch-regulated c-Kit−positive liver sinusoidal endothelial cells contribute to liver zonation and regeneration. Cell Mol Gastroenterol Hepatol 2022;13:1741–56.
67. Almanzar N, Antony J, Baghel AS, et al. A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. Nature 2020;583:590–5.
68. Shimizu S, Iba T, Naito H, et al. Aging impairs the ability of vascular endothelial stem cells to generate endothelial cells in mice. Angiogenesis 2023;26:567–80.
69. Brack AS, Bildsoe H, Hughes SM. Evidence that satellite cell decrement contributes to preferential decline in nuclear number from large fibres during murine age-related muscle atrophy. J Cell Sci 2005;118:4813–21.
70. Ibrayeva A, Bay M, Pu E, et al. Early stem cell aging in the mature brain. Cell Stem Cell 2021;28:955–966.e7.
71. Dykstra B, Olthof S, Schreuder J, Ritsema M, De Haan G. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med 2011;208:2691–703.
72. Hong X, Campanario S, Ramírez-Pardo I, Grima-Terrén M, Isern J, Muñoz-Cánoves P. Stem cell aging in the skeletal muscle: The importance of communication. Ageing Res Rev 2022;73:101528.
73. Wang WM, Xu XS, Miao CM. Kupffer cell-derived TNF-α triggers the apoptosis of hepatic stellate cells through TNF-R1/caspase 8 due to ER stress. Biomed Res Int 2020;2020:8035671.
74. Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G. Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. FASEB J 2003;17:1183–5.
75. Lee C-K, Allison DB, Brand J, Weindruch R, Prolla TA. Transcriptional profiles associated with aging and middle age-onset caloric restriction in mouse hearts. Proc Natl Acad Sci U S A 2002;99:14988–93.
76. Belmin J, Bernard C, Corman B, Merval R, Esposito B, Tedgui A. Increased production of tumor necrosis factor and interleukin-6 by arterial wall of aged rats. Am J Physiol 1995;268:H2288–93.
77. Bruunsgaard H, Skinhøj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, tumour necrosis factor-alpha (TNF-α) and atherosclerosis. Clin Exp Immunol 2000;121:255–60.
78. Naito H, Iba T, Wakabayashi T, et al. TAK1 Prevents endothelial apoptosis and maintains vascular integrity. Dev Cell 2019;48:151–166.e7.
79. Gou B, Chu X, Xiao Y, et al. Single-cell analysis reveals transcriptomic reprogramming in aging cardiovascular endothelial cells. Front Cardiovasc Med 2022;9:900978.