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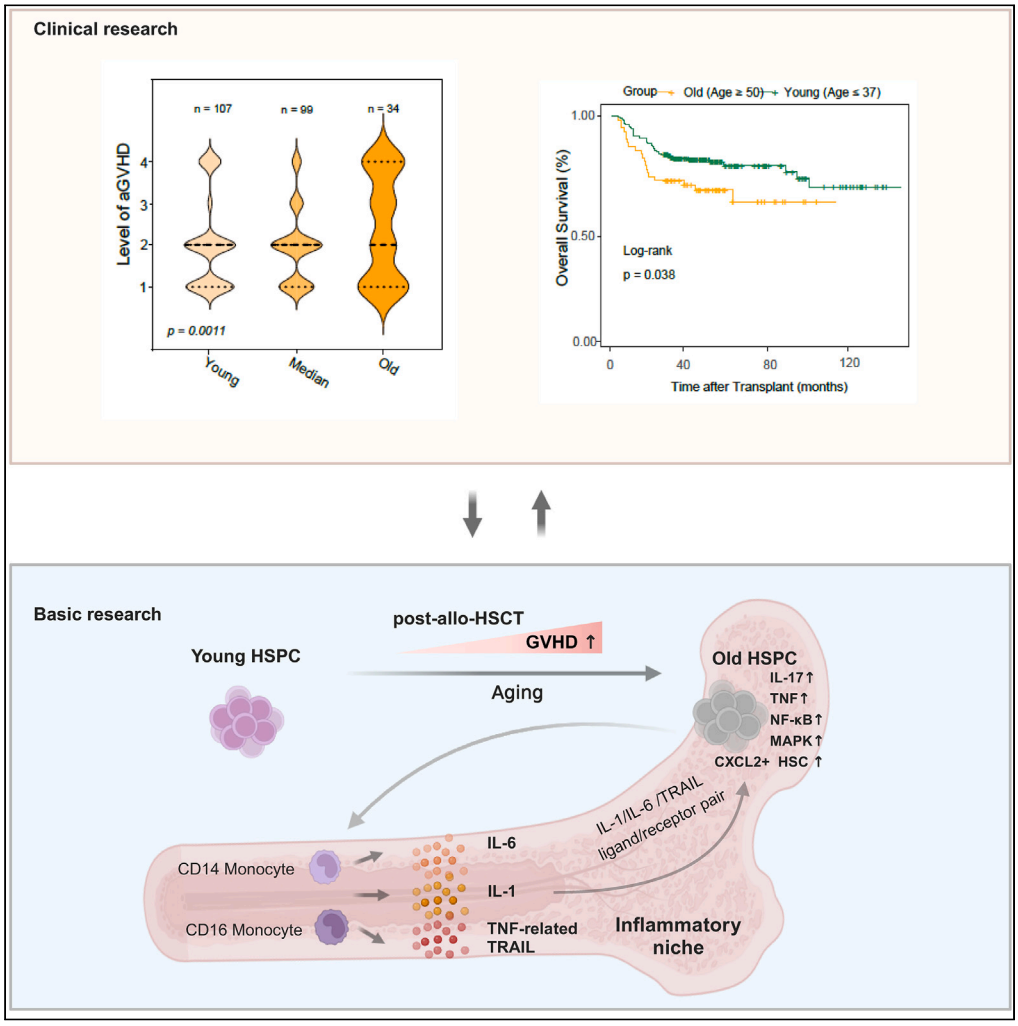

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Highlights

Old donors in allogeneic HSCT are prone to increased severity of aGVHD and poor OS

Aged HSCs display increased inflammatory pathways and differentiation dysfunction

Monocyte-derived IL-1, IL-6, and TNF mainly contribute to HSC aging

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Monocytes in allo-HSCT with aged donors secrete IL-1/IL-6/TNF to increase the risk of GVHD and damage the aged HSCs

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SUMMARY

Aging is considered a critical factor of poor prognosis in allogenic hemopoietic stem cell transplantation (allo-HSCT). To elucidate the underlying mechanisms, we comprehensively reintegrated our clinical data from patients after allo-HSCT and public single-cell transcriptomic profile from post-allo-HSCT and healthy individuals, demonstrating that old donors were more prone to acute GVHD (aGVHD) with pronounced inflammation accumulation and worse overall survival (OS). We also found the presence of inflammation-related CXCL2+ HSC subpopulation during aging with significantly enriched pro-inflammatory pathways. Shifting attention to the HSC microenvironment, we deciphered that IL-1/IL-6 and TRAIL (i.e., TNFSF10) ligand–receptor pair serves as the crucial bridge between CD14/CD16 monocytes and hematopoietic stem/progenitor cells (HSPCs). The profound upregulation of these signaling pathways during aging finally causes HSC dysfunction and lineage-biased differentiation. Our findings provide the theoretical basis for achieving tailored GVHD management and enhancing allo-HSCT regimens efficacy for aged donors.

INTRODUCTION

Since 1957, hematopoietic stem cell transplantation (HSCT) has been considered as a potentially curative therapeutic option for many hemopathies.[1] However, graft-versus-host disease (GVHD), accompanied by multi-organ inflammation, is one of the most significant complications and a major reason for HSCT failures.[2] To date, many studies have reported that older donors have a poor prognosis of HSCT, including a higher risk of GVHD and lower overall survival (OS). For example, in the T cell receptor (TCR) haploidentical transplantation system, the Beijing protocol states that donor age >30 years presents higher non-relapse mortality (NRM) and lower OS than the ≤30 years group.[3] Further studies in a total of 1,270 patients including T cell depletion (TCD) and TCR haploidentical transplantation noted that donor age affected HSCT outcome in acute leukemia patients >40 years old, with a higher risk of NRM related to aged donors (age >40 years old).[4]

The mechanisms underlying poor OS and higher GVHD incidence with aged-donor-derived stem cells in haploidentical transplantation systems remain elusive. This phenomenon may be related to the aging dynamics of donor-derived HSCs and HSCT niche. In TCD HSCT, using younger donors is related to improved immune recovery in CD4 and B lymphocyte populations as well as a lower rate of acute GVHD.[5] In HSCT allografts, $CD34^+$ cells depletions may be a critical factor associated with delayed platelet engraftment after unmanipulated haploidentical transplantation.[6] Furthermore, early $CD4^+$ IR, a simple and robust predictive marker of HCT outcomes, is associated with survival after acute GVHD.[7] The inflammatory factor and inflammation signaling from the HSC bone marrow niche or GVHD procedure in HSCT in turn affected the activity and function of hematopoietic stem cells (HSCs).[8,9]

As public data from single-cell RNA sequencing (scRNA-seq) increases dramatically, we reintegrated patient data in HSC niche after allo-HSCT (GSE224714)[10] as well as healthy human data of HSCs (GSE104379)[11] and HSC niche (GSE120221) during aging.[12] The data in GSE104379 highlight that downregulation of genes epigenetically altered with age leads to impaired HSC differentiation, whereas the data in GSE120221 provided a comprehensive assessment of human bone marrow cells using both scRNA-seq and multiparameter flow cytometry from 20 healthy adult human donors across a broad age range. To decipher the underlying correlations between donor-age-dependent acute

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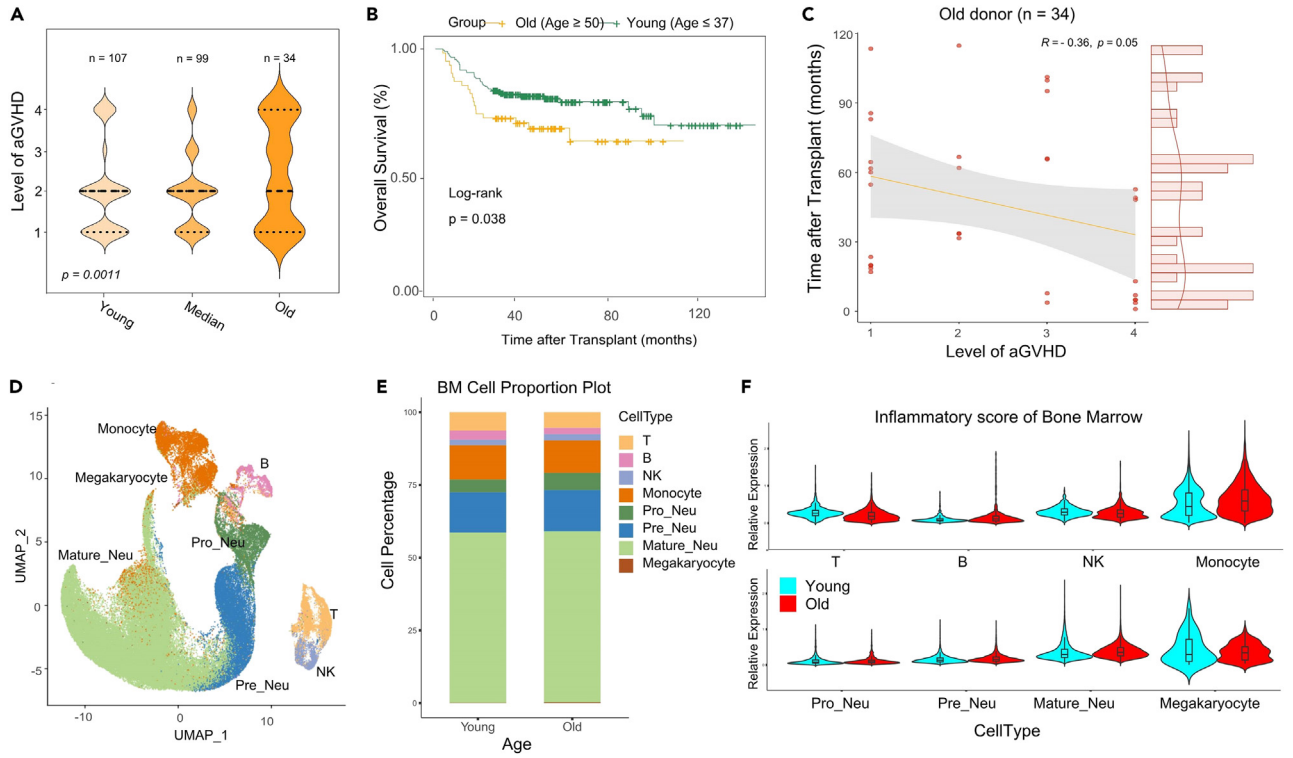

Figure 1. The positive correlation between donor age and the severity of aGVHD, poor OS, and inflammation accumulation in allo-HSCT

(A) Violin plots revealing trends in the severity of aGVHD (one-way ANOVA, p = 0.0005) during donors aging.

(B) Kaplan–Meier curves of OS after allo-HSCT according to the age of donors (log rank test, p = 0.038).

(C) Correlations between OS after allo-HSCT and level of aGVHD in old donors (Spearman test, p = 0.026).

(D) The uMAP of allo-HSCT patients data marked by different clusters.

(E) Cluster proportions in patients' BM, who receive HSCT from young and old donors.

(F) Violin plot of inflammation scores in each cluster in BM of transplant patients receiving from young and old donors. n represents number of corresponding patients. aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; OS, overall survival; allo-HSCT, allogenic hematopoietic stem cell transplantation; BM, bone marrow. The detailed inflammation-related gene list is in Table S1. See also Figure S1.

GVHD (aGVHD) and limited overall survival (OS) of patients after HSCT, the present study aimed to illustrate the effects of inflammation on HSC dysfunction and aging.

RESULTS

Donor age positively correlated with aGVHD severity, lower OS, and inflammatory scores upregulation after allo-HSCT

To identify how GVHD severity and OS relate to donor age, we investigated the data of 443 patients treated with allo-HSCT in our center. Based on the optimal cut point principle and clinical practicality, the donor age was divided into three groups: young (age ≤ 37 years), medium (age 38–49 years), and old (age ≥ 50 years). Increased donor age was significantly correlated with the severity of aGVHD (p = 0.0005) but not chronic GVHD (cGVHD) (p = 0.23) (Figures 1A and S1A). OS was also significantly different between the young and old groups (Figure 1B; p = 0.038). Furthermore, the severity of aGVHD was significantly correlated with OS in old (Figure 1C; p = 0.026) donors.

To delineate the potential relationship between the aged donor and aGVHD after HSCT, we analyzed the single-cell transcriptomic data of bone marrow hematopoietic reconstitution process from four representative patients with HSCT. Two patients accepting aged bone marrow experienced severe GVHD, whereas the other two with young donors had mild symptoms. The corresponding 50,519 cells from GSE224714 dataset were annotated to eight clusters including T, B, NK, monocyte, pro_neu (progenitor of neutrophil), pre_neu (precursor of neutrophil), mature_neu (mature neutrophil), and megakaryocyte (Figure 1D). Canonical markers (CD3D, CD19, CD79A, NKG7, PRF1, CD14, CD24, LTF, FCGR3B, CXCR2, and PF4) were used to confirm annotation robustness (Figure S1B). No significant difference in cell-type proportion was observed between the patients with young and old donors (Figure 1E).

The inflammatory response represents a critical aspect of GVHD. To pinpoint the cell subpopulation potentially responsible for the distinct GVHD pathogenesis associated with elderly donors, we used the hallmark inflammatory response pathway to assess single-cell inflammatory characteristics (Table S1). Of all the cell types, monocytes displayed the most pronounced increase in inflammatory response among patients

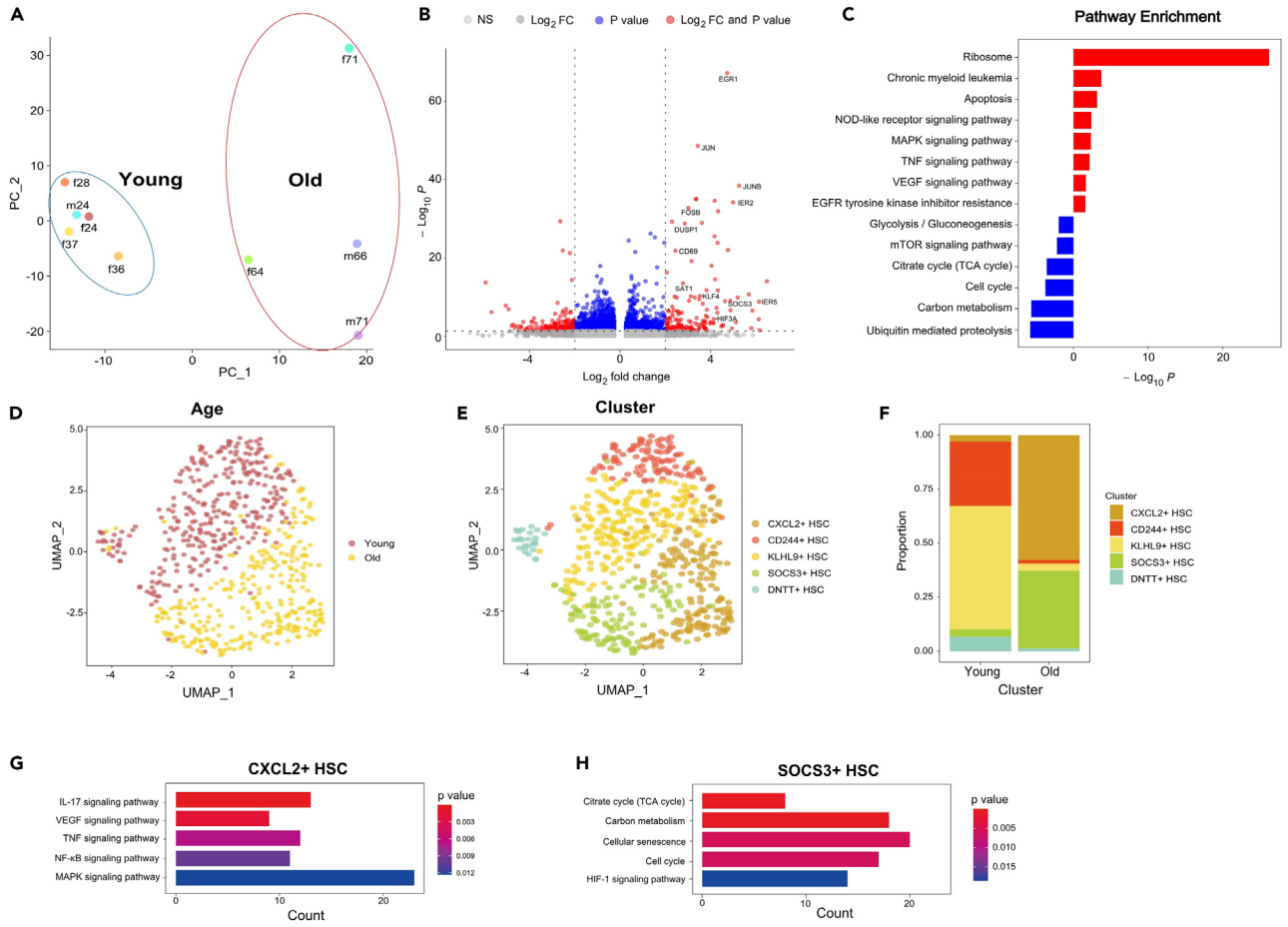

Figure 2. Identification of cellular heterogeneity of HSCs during aging

(A) PCA plot for the averaged position of all cells in each donor.

(B) Volcano plot illustrating differentially expressed genes. The detailed gene list is in Table S2.

(C) The barplot presenting the KEGG pathway enrichments in the whole dataset.

(D) UMAP plots of the HSC single-cell dataset with ages and clusters (E).

(F) Cluster proportion in young and old donors, respectively.

(G) Barplots illustrating upregulated KEGG pathway enriched in CXCL2+ and SOCS3+ HSC (H). See also Figure S2.

with aged donors. This observation underscores the significant role played by monocytes in the pathogenesis of age-related GVHD in donors (Figure 1F).

Inflammation triggers HSC aging and lineage-biased differentiation

HSCs play a pivotal role in the hematopoietic reconstitution following HSCT. Consequently, to decipher HSC transcriptional heterogeneity during aging, we analyzed 650 HSCs from 9 healthy donors from the GSE104379 dataset.

After strict quality control and filtration, a total of 622 HSCs with 16,988 genes were obtained and divided into two groups: young (age $\leq$37 years) and old ($\geq$60 years old). We characterized the increased heterogeneity between individuals during aging (Figure 2A), encompassing notably upregulated genes in aged HSCs compared with young ones, such as JUN, FOSB, DUSP1, KLF4, SOCS3, and HIF3A (Figure 2B; Table S2). To reveal the change in key signaling pathways in HSCs during aging, we performed GSEA functional enrichment analysis and found that upregulated genes were enriched in several inflammation-related pathways (e.g., mitogen-activated protein kinase [MAPK] signaling pathway, tumor necrosis factor (TNF) signaling pathway, and NOD-like receptor signaling pathway) (Figure 2C). Furthermore, we found that classical metabolism-related pathways in senescent HSCs, including glycolysis/gluconeogenesis, citrate cycle (TCA cycle), and carbon metabolism, were deregulated.

To further investigate HSC heterogeneity, dimensionality reduction was performed. All samples were well integrated: cells from different genders and ages were interspersed across distinct clusters, and all clusters contained cells from multiple samples (Figures 2D and S2A–S2E). Donor age versus gender stratification showed a more significant effect on HSC heterogeneity, which was in line with previous findings

(Figures 2D and S2E). Meanwhile, visualizing the t-distributed stochastic neighbor embedding (tSNE) result also revealed a tight distribution of data points for old donor cells and male donor cells, whereas a disorganized distribution of data points for female donor cells and young and medium donor cells (Figure S2B). These results suggest that age might contribute more to the heterogeneity of human HSCs in bone marrow than gender.

Furthermore, we identified five cell clusters: CXCL2+, CD244+, KLHL9+, SOCS3+, and DNTT+ HSCs (Figures 2E and S2F). The percentages of CXCL2+ and SOCS3+ HSC increased in the aged groups, whereas CD244+, KLHL9+, and DNTT+ HSC percentages decreased (Figure 2F). The top markers of the CXCL2+ HSC cluster were enriched in inflammatory pathways, including IL-17, vascular endothelial growth factor (VEGF), TNF, nuclear factor κB (NF-κB), and MAPK signaling pathways (Figure 2G), whereas those of the SOCS3+ HSC cluster were enriched in metabolic pathways such as the TCA cycle, carbon metabolism, cellular senescence, cell cycle, and HIF signaling pathways (Figure 2H).

Single-cell transcriptomic landscape of bone marrow niche during aging

The dynamic and complex interactions between cell types in the bone marrow niche and HSPC/HSCs play a critical role in HSC aging. To illustrate the dynamic change in the HSC niche during aging, UMAP was performed on bone marrow cells, and all samples were well integrated: cells from different ages and genders were interspersed across distinct clusters, and all clusters contained cells from multiple samples (Figures 3A and S3A). All 16 samples were divided into three groups: young (age $\leq$ 37 years), median (age 38–59 years), and old ($\geq$60 years old). Based on the annotated subsets and prior knowledge, 48,104 cells were divided into 11 different clusters (Figure 3A). Canonical markers of different cell types confirmed annotation robustness: CD34 for HSPCs, CD8A and PRF1 for CD8 T cells, KLRD1 for natural killer (NK) cells, CD79A for B cells, DERL3 and CSTA for plasma cells, CD14 for CD14 monocytes, FCGR3A for CD16 monocytes, IRF4/8 for DCs, and SLC2A1 for erythrocytes (Figures S3B and S3C). Distinct UMAP visualization of 16 bone marrow samples illustrated high inter-sample heterogeneity (Figure S3D). Overall, despite considerable patient heterogeneity, only naive CD8 T cells showed pronounced significant cellular composition changes in bone marrow subsets during aging (Figure S3E).

CD14/CD16 monocytes in aged bone marrow niche contribute most to inflammation accumulation and GVHD risk

To further reveal the relationship between the different bone marrow cell types and GVHD, we analyzed KEGG and GVHD scores. KEGG results showed that CD14 monocytes, CD16 monocytes, and HPSCs exhibited significant changes in inflammation-related signaling pathways during aging (Figure 3B). For instance, GVHD, NF-κB signaling pathway, and MAPK signaling pathway were pronounced in aged CD14 monocytes. Notably, the GVHD-related signaling pathways exhibited widespread upregulation in the aforementioned three clusters, which emphasized that the bone marrow microenvironment in older patients is more susceptible to GVHD.

These results may account for the positive correlation between donor age and aGVHD severity after HSCT in the clinic. To determine the contribution of each cell subset in GVHD and inflammation, we next sought to compute the GVHD and inflammation score for each cell type as the mean expression level of 198 GVHD-related and 200 inflammation-related genes (Tables S1 and S3). The GVHD score of CD4 T cells, CD8 T cells, NK cells, CD14 monocytes, CD16 monocytes, and DCs changed significantly ($p < 0.05$), whereas the inflammation score of HSPCs, CD4 T cells, CD8 T cells, B cells, CD14 monocytes, CD16 monocytes, and DCs changed significantly ($p < 0.05$), further indicating their crucial role in GVHD and inflammatory HSC niche (Figure 3C).

Inflammatory IL-1/IL-6/TNF signaling from CD14/CD16 monocytes is a crucial bridge between monocytes and HSPC/HSCs

To elucidate the dynamic regulatory network of HSC function in bone marrow during aging, cell-cell interaction inference analysis was performed with CellChat. The results of pathways targeting HSPCs showed that IL-1, IL-6, TRAIL (i.e., TNFSF10), LIGHT, and FGF signaling pathways presented widespread upregulation in old donor group, whereas FLT3, BAFF, and NPR2 were also upregulated in the young donor group (Figure 4A). Based on the ligand-receptor interaction number, global cellular communication of immune cells targeting HSPCs gradually strengthens during aging, especially the CD14 monocyte-HSPC axis (Figure 4B). Additionally, the crucial intersection in three significantly changed signaling pathways (MAPK, TNF, and Nod-like signaling pathways) in HSCs during aging contained IL-1B, TAB2, TNF, NFKB1, and TRAF1 (Figures 2A–2C and 4C). Additionally, the notable changes in specific ligand-receptor interactions during aging were IL-1 and IL-6 pathways in the CD14 monocyte-HSPC axis. The most varied ligand-receptor pairs in the IL-1 pathway were IL-1B-(IL-1R1+IL-1RAP/IL-1R2) and IL-6-(IL-6R + IL-6ST) in IL-6 pathway (Figures 4D and 4E). Meanwhile, TRAIL pathways in the CD16 monocytes also exhibited a strong inter cellular interaction with HSPCs via TNFSF10-TNFRSF10B ligand-receptor pairs. These results suggest that the inflammatory factors, paracrine IL-1/IL-6 and TNFSF10 from senescent CD14 and CD16 monocytes, respectively, and HSPC autocrine, constantly activate the MAPK and TNF signaling pathways in HSPCs, finally resulting in the dysfunction of HSC and subsequent lineage-biased differentiation.

To further investigate the heterogeneity and internal changes in CD14 and CD16 monocytes, they were reclustered. After unsupervised dimensionality reduction, three subpopulations—IL-1B+ CD14 monocytes and RETN+ CD14 monocytes—were identified in the CD14 monocyte cluster (Figures 4H and 4I), whereas VMO1+, IL1B+, and TNF+ subpopulations were identified in CD16 monocytes (Figures 4K and 4L). The increased proportion of IL-1B+ CD14 and CD16 monocytes during aging revealed the crucial role of CD14 and CD16 monocytes in age-related HSC dysfunction (Figures 4J and 4M). Notably, although the proportion of TNF+ CD16 monocytes decreases with age, there is a significant increase in CD16 monocytes with considerable expression of TNFSF10 compared with all myeloid cells, suggesting the importance of TNF-family-involved pathways.

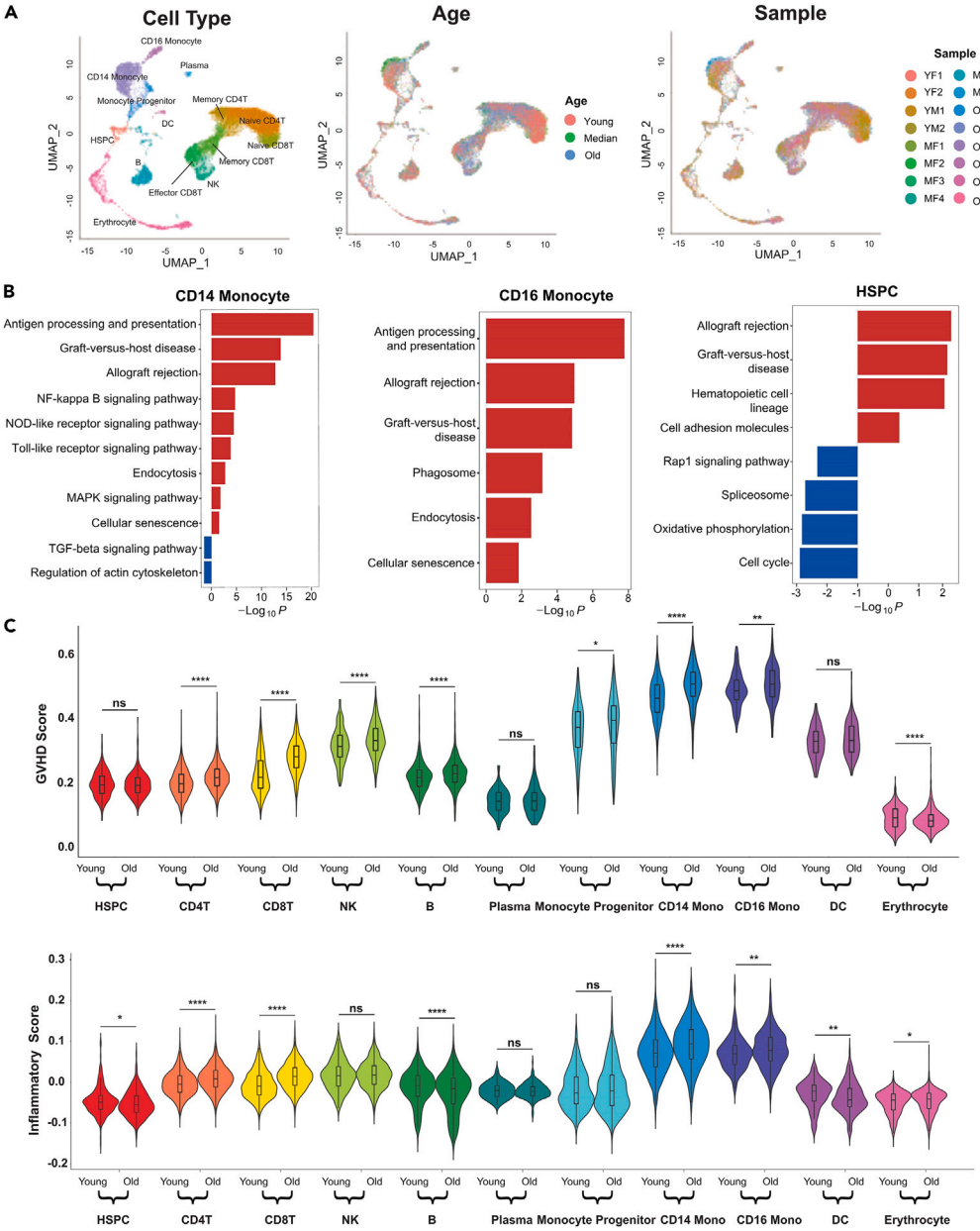

Figure 3. Functional changes of different clusters in bone marrow niche during aging

(A) UMAP plots of HSPC bone marrow niche with colors indicating different ages, samples, and clusters.

(B) Plots of the significantly enriched pathways via GSEA in CD4 T cells, CD8 T cells, NK cells, CD14 monocytes, CD16 monocytes, and HSPCs.

(C) Violin plot of GVHD and inflammation scores in each cluster during aging. Wilcoxon test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001; *ns*, no significance. See also Figure S3 and Table S3. The GVHD-related gene list is in Table S3, and the inflammation-related gene list is in Table S1. See also Figure S3.

DISCUSSION

aGVHD is one of the most significant complications of HSCT accompanied by multi-organ inflammation. In the clinic, donor age is positively correlated with the severity of aGVHD and low OS after HSCT (Figures 1A–1D). It is highly consistent with the original findings that the older donor group had a higher incidence of aGVHD with poor OS.[13] To illustrate the role of transplanted HSC products from old donors in the occurrence of aGVHD, we explored the cellular dynamics in the bone marrow from patients treated with allo-HSCT. We also analyzed HSC and HSC niche characterization and their contribution to GVHD and inflammation accumulation during donor aging.

Differentially expressed genes (DEGs) between young and aged HSCs and their biological functions were extensively explored. For example, the specific differential expression gene Klf4 between aged and young HSCs (Figure 2B) is a known inhibitor of HSC self-renewal.[14]

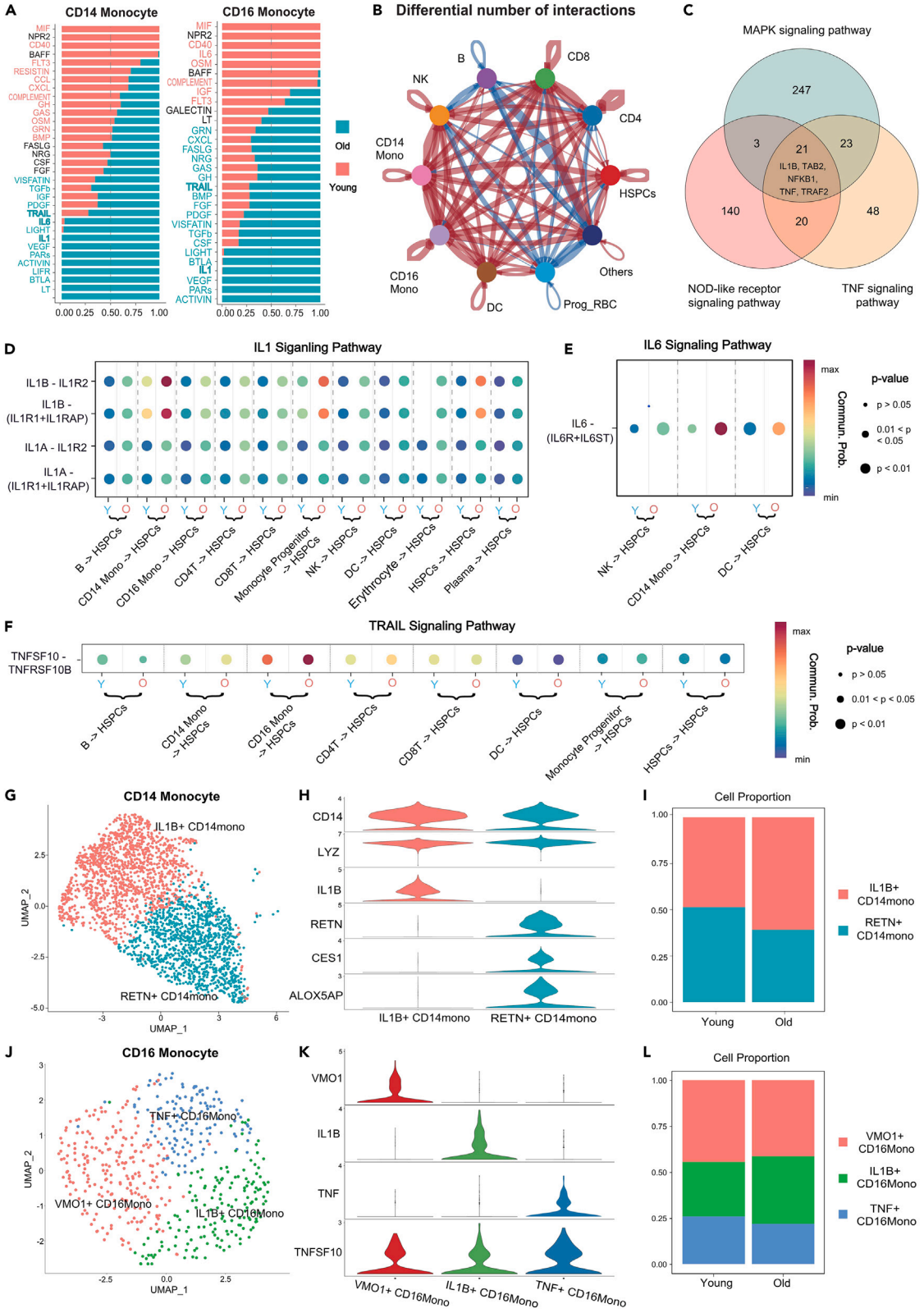
A
CD14 Monocyte
CD16 Monocyte
Old
Young
B
Differential number of interactions
B
CD8
NK
CD4
CD14 Mono
HSPCs
CD16 Mono
Others
DC
Prog_RBC
C
MAPK signaling pathway
247
3
21
23
IL1B, TAB2, NFKB1, TNF, TRAF2
140
20
48
NOD-like receptor signaling pathway
TNF signaling pathway
D
IL1 Siganling Pathway
IL1B - IL1R2
IL1B - (IL1R1+IL1RAP)
IL1A - IL1R2
IL1A - (IL1R1+IL1RAP)
B -> HSPCs
CD14 Mono -> HSPCs
CD16 Mono -> HSPCs
CD4T -> HSPCs
CD8T -> HSPCs
Monocyte Progenitor -> HSPCs
NK -> HSPCs
DC -> HSPCs
Erythrocyte -> HSPCs
HSPCs -> HSPCs
Plasma -> HSPCs
E
IL6 Signaling Pathway
IL6 - (IL6R+IL6ST)
NK -> HSPCs
CD14 Mono -> HSPCs
DC -> HSPCs
max
min
Commun. Prob.
p-value
p > 0.05
0.01 < p < 0.05
p < 0.01
F
TRAIL Signaling Pathway
TNFSF10 - TNFRSF10B
B -> HSPCs
CD14 Mono -> HSPCs
CD16 Mono -> HSPCs
CD4T -> HSPCs
CD8T -> HSPCs
DC -> HSPCs
Monocyte Progenitor -> HSPCs
HSPCs -> HSPCs
G
CD14 Monocyte
JL1B+ CD14mono
RETN+ CD14mono
UMAP_1
UMAP_2
H
CD14
LYZ
IL1B
RETN
CES1
ALOX5AP
IL1B+ CD14mono
RETN+ CD14mono
I
Cell Proportion
Young
Old
IL1B+ CD14mono
RETN+ CD14mono
J
CD16 Monocyte
TNF+ CD16Mono
VMO1+ CD16Mono
IL1B+ CD16Mono
UMAP_1
UMAP_2
K
VMO1
IL1B
TNF
TNFSF10
VMO1+ CD16Mono
IL1B+ CD16Mono
TNF+ CD16Mono
L
Cell Proportion
Young
Old
VMO1+ CD16Mono
IL1B+ CD16Mono
TNF+ CD16Mono

Figure 4. Inflammatory pathways serve as the crucial bridge between CD14/CD16 monocytes and HSPCs

(A) Signaling pathways in the overall information flow between young and old groups. The red bar represents the young group, and the blue bar represents the old group. The signaling pathways in red texts were upregulated in the young group, and the pathways in blue texts were upregulated in the old group.

(B) Circle plot of the interaction numbers among different cell types. The red line represents the higher interaction number in the old group. The blue line represents the higher interaction number in the young group.

(C) Venn diagram of genes in three significant changed signaling pathways during HSC aging.

(D) Bubble plot of each cell cluster targeting HSPCs in IL-1 signaling pathway, IL-6 signaling pathway (E), and TRAIL signaling pathway (F).

(G) The UMAP of CD14 monocyte clusters.

(H) Violin plot of the top markers in different CD14 monocyte subpopulations.

(I) Barplot of the cellular composition of CD14 monocyte cluster in the young and old groups.

(J) The UMAP of CD16 monocyte clusters.

(K) Violin plot of the top markers in different CD16 monocyte subpopulations.

(L) Barplot of the cellular composition in CD16 monocyte cluster in the young and old groups.

Furthermore, Klf6 is involved in inflammation and myeloid differentiation,[15,16] whereas Klf5 enhances age- and inflammation-related LT-HSC myeloid bias.[17] These results demonstrate the effects of the KLF family on HSCs.

Furthermore, our studies revealed genes upregulated in the aging HSC, which were primarily involved in inflammatory pathways, including TNF, MAPK, and NOD-like receptors. These inflammatory response pathways enriched in aged HSCs are consistent with previous findings, which were mainly correlated with inflammatory response and STAT3/IFNα/β targets.[11] The subgroups sensitive to inflammatory pathways were mainly CXCL2+ HSC subpopulations, whose proportion increased during aging and DEGs of which were mainly enriched in TNF, NF-Kβ, and MAPK pathways (Figures 2G and 2H). These results suggested that the CXCL2+ HSC population migrates to the inflammatory sections during aging. On the other hand, the genes downregulated in aging HSCs were enriched in the metabolic pathways (including glycolysis and TCA cycle) (Figure 2C), and their corresponding subset was mainly SOCS3+ HSCs. Meanwhile, the proportion of SOCS3+ HSCs subpopulations decreased with donor age, and their DEGs were highly enriched in the TCA cycle, carbon metabolism, and cellular senescence. Additionally, in line with this, previous studies have shown that the HSPC subset with high glucose uptake is highly enriched for HSPC with a differentiation bias toward the myeloid lineages.[18] In 2022, Suo et al. also found that Igf2bp2 at a young age regulates the metabolism- and protein-synthesis-related genes necessary for the complete function of HSCs in young adult mice.[19] These aging-related inflammatory features of HSCs may partly explain the higher GVHD risk of patients with aged donors after HSCT. These inflammatory features are closely related to the functional decline of HSCs, such as the differentiation bias and decreased self-renewal ability, which may impair normal hematopoietic reconstitution after HSCT.[20] In addition, such aging-related state may be accompanied by the epigenetic landscape reshaping, which is inherited by downstream progenitors or mature leukocytes causing dysfunctional bone marrow as well as GVHD.[21]

In addition to HSCs, we identified cell clusters of the HSC bone marrow niche, calculated a GVHD and inflammation score of each subpopulation (Figure 3C), and analyzed intercellular communication between HSPCs and other cell types (Figure 4), highlighting the contributions of IL-1B+ CD14/CD16 monocytes. In 2022, Caiado et al. demonstrated that increased IL-1 levels in the bone marrow during aging drove $Tet2^{+/-}$ clonal expansion via increased HSPC proliferation and multilineage differentiation. Furthermore, genetic deletion of IL-1R1 abolishes and pharmacological inhibition of IL-1-IL-1R1 signaling impairs $Tet2^{+/-}$ clonal expansion during aging.[22] For IL-1 source, other than IL-1B + CD14 monocytes, Kovtonyuk et al. found that increased blood microbial compounds also drive elevated IL-1A/B production in the bone marrow of older mice. HSCs in older IL-1R1 KO and germ-free mice are protected from HSC inflammation, whereas IL-1 antagonist or antibiotic treatment reverts HSC inflammation.[23] Furthermore, previous findings indicated that the amounts of monocytes in allografts between young and old donors displayed profound correlation with the adverse prognosis of patients with GVHD.[24] These phenomena highlight the important roles and potential mechanism of monocyte-derived IL-1 in HSC clonal expansion and myeloid bias differentiation.

Furthermore, we elucidated that TNF family also involves in this process, as the significantly increased TNF+ CD16 monocytes and TNFSF10-TNFRSF10B ligand-receptor pairs from CD16 monocytes to HSPCs. However, Yamashita and Passegué proposed that upregulated TNFα during aging may serve as a vital pro-survival and regeneration factor for HSCs (2019).[25]

Collectively, our study elucidated the biological characteristics of CD14-/CD16-monocytes-related IL-1, CD14-monocytes-related IL-6, and CD16-monocytes-related TNF family during aging and revealed their potential contribution to aGVHD and HSC dysfunction. To date, the roles of IL-27α,[26] IFN-α,[27] IFN-γ,[28] and IL-1β[22,23] in HSCs have been reported in mouse models. Although the effect of inflammation on HSCs has been widely accepted, we speculated that GVHD-related genes IL-1, IL-6, and TNF family, pivotal proinflammatory factors, mainly mediate the dysfunction of HSC in patients with HSCT. Moreover, the genes and pathways we identified could be potential biomarkers and targets for functional rejuvenation of aged HSC.

Limitations of the study

Although we provided valuable insights into the underlying mechanisms of poorer prognosis and higher aGVHD levels in allo-HSCT with older donor, further preclinical experiments and large-cohort clinical trials blocking monocyte-derived IL-1/IL-6/TNF pathways in both young and old HSCs would provide more direct evidence on the proposed mechanisms.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109126.

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AUTHOR CONTRIBUTIONS

X.L., W.Z., Y.W., and C.L. conceived the study, did the single-cell data analysis, and wrote the original draft. Y.W., X.L., and Y.L. counted and provided the clinical data of GVHD patients after allo-HSCT. Y.S., Y.L., Y.W., X.Z., and Z.C. revised the original manuscript and figures. Y.L., P.Q., and H.H. participated in the discussion of the whole manuscript, and finally approved the manuscript for submission.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1. Thomas, E.D., Lochte, H.L., Jr., Lu, W.C., and Ferrebee, J.W. (1957). Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy. N. Engl. J. Med. *257*, 491–496. https://doi.org/10.1056/nejm195709122571102.
2. Gooley, T.A., Chien, J.W., Pergam, S.A., Hingorani, S., Sorror, M.L., Boeckh, M., Martin, P.J., Sandmaier, B.M., Marr, K.A., Appelbaum, F.R., et al. (2010). Reduced mortality after allogeneic hematopoietic-cell transplantation. N. Engl. J. Med. *363*, 2091–2101. https://doi.org/10.1056/NEJMoa1004383.
3. Wang, Y., Chang, Y.J., Xu, L.P., Liu, K.Y., Liu, D.H., Zhang, X.H., Chen, H., Han, W., Chen, Y.H., Wang, F.R., et al. (2014). Who is the best donor for a related HLA haplotype-mismatched transplant? Blood *124*, 843–850. https://doi.org/10.1182/blood-2014-03-563130.
4. Canaani, J., Savani, B.N., Labopin, M., Huang, X.J., Ciceri, F., Arcese, W., Koc, Y., Tischer, J., Blaise, D., Gülbas, Z., et al. (2018). Donor age determines outcome in acute leukemia patients over 40 undergoing haploidentical hematopoietic cell transplantation. Am. J. Hematol. *93*, 246–253. https://doi.org/10.1002/ajh.24963.
5. González-Vicent, M., Molina, B., Deltoro, N., Sevilla, J., Vicario, J.L., Castillo, A., Ramirez, M., and Díaz, M.Á. (2017). Donor age matters in T-cell depleted haploidentical hematopoietic stem cell transplantation in pediatric patients: Faster immune reconstitution using younger donors. Leuk. Res. *57*, 60–64. https://doi.org/10.1016/j.leukres.2017.03.001.
6. Chang, Y.J., Xu, L.P., Liu, D.H., Liu, K.Y., Han, W., Chen, Y.H., Yu, W., Chen, H., Wang, J.Z., Zhang, X.H., et al. (2009). Platelet engraftment in patients with hematologic malignancies following unmanipulated haploidentical blood and marrow transplantation: effects of CD34+ cell dose and disease status. Biol. Blood Marrow Transplant. *15*, 632–638. https://doi.org/10.1016/j.bbmt.2009.02.001.
7. de Koning, C., Prockop, S., van Roessel, I., Kernan, N., Klein, E., Langenhorst, J., Szanto, C., Belderbos, M., Bierings, M., Boulad, F., et al. (2021). CD4+ T-cell reconstitution predicts survival outcomes after acute graft-versus-host-disease: a dual-center validation. Blood *137*, 848–855. https://doi.org/10.1182/blood.2020007905.
8. Hurwitz, S.N., Jung, S.K., and Kurre, P. (2020). Hematopoietic stem and progenitor cell signaling in the niche. Leukemia *34*, 3136–3148. https://doi.org/10.1038/s41375-020-01062-8.
9. Pinho, S., and Frenette, P.S. (2019). Haematopoietic stem cell activity and interactions with the niche. Nat Rev Mol Cell Biol *20*, 303–320. https://doi.org/10.1038/s41580-019-0103-9.
10. Huo, Y., Wu, L., Pang, A., Li, Q., Hong, F., Zhu, C., Yang, Z., Dai, W., Zheng, Y., Meng, Q., et al. (2023). Single-cell dissection of human hematopoietic reconstitution after allogeneic hematopoietic stem cell transplantation. Sci. Immunol. *8*, eabn6429. https://doi.org/10.1126/sciimmunol.abn6429.
11. Adelman, E.R., Huang, H.T., Roisman, A., Olsson, A., Colaprico, A., Qin, T., Lindsley, R.C., Bejar, R., Salomonis, N., Grimes, H.L., and Figueroa, M.E. (2019). Aging Human Hematopoietic Stem Cells Manifest Profound

Epigenetic Reprogramming of Enhancers That May Predispose to Leukemia. Cancer Discov. *9*, 1080–1101. https://doi.org/10.1158/2159-8290.Cd-18-1474.

12. Oetjen, K.A., Lindblad, K.E., Goswami, M., Gui, G., Dagur, P.K., Lai, C., Dillon, L.W., McCoy, J.P., and Hourigan, C.S. (2018). Human bone marrow assessment by single-cell RNA sequencing, mass cytometry, and flow cytometry. JCI insight *3*, e124928. https://doi.org/10.1172/jci.insight.124928.
13. Arai, Y., Kondo, T., Yamazaki, H., Takenaka, K., Sugita, J., Kobayashi, T., Ozawa, Y., Uchida, N., Iwato, K., Kobayashi, N., et al. (2016). Allogeneic unrelated bone marrow transplantation from older donors results in worse prognosis in recipients with aplastic anemia. Haematologica *101*, 644–652. https://doi.org/10.3324/haematol.2015.139469.
14. Heidel, F.H., Bullinger, L., Arreba-Tutusaus, P., Wang, Z., Gaebel, J., Hirt, C., Niederwieser, D., Lane, S.W., Döhner, K., Vasioukhin, V., et al. (2013). The cell fate determinant Llgl1 influences HSC fitness and prognosis in AML. J. Exp. Med. *210*, 15–22. https://doi.org/10.1084/jem.20120596.
15. Kim, G.D., Das, R., Goduni, L., McClellan, S., Hazlett, L.D., Mahabeleshwar, G.H., Keller, G., and Friedman, S.L. (2006). Developmental regulation of yolk sac hematopoiesis by Kruppel-like factor 6. J. Biol. Chem. *107*, 21271–21282. https://doi.org/10.1182/blood-2005-05-1916.
16. Kim, G.D., Das, R., et al. (2016). Kruppel-like Factor 6 Promotes Macrophage-mediated Inflammation by Suppressing B Cell Leukemia/Lymphoma 6 Expression. J Biol Chem *291*, 21271–21282. https://doi.org/10.1074/jbc.M116.738617.
17. Mann, M., Mehta, A., de Boer, C.G., Kowalczyk, M.S., Lee, K., Haldeman, P., Rogel, N., Knecht, A.R., Farouq, D., Regev, A., and Baltimore, D. (2018). Heterogeneous Responses of Hematopoietic Stem Cells to Inflammatory Stimuli Are Altered with Age. Cell Rep. *25*, 2992–3005.e5. https://doi.org/10.1016/j.celrep.2018.11.056.
18. Poisa-Beiro, L., Landry, J.J.M., Raffel, S., Tanaka, M., Zaugg, J., Gavin, A.C., and Ho, A.D. (2022). Glucose Metabolism and Aging of Hematopoietic Stem and Progenitor Cells. Int. J. Mol. Sci. *23*, 3028. https://doi.org/10.3390/ijms23063028.
19. Suo, M., Rommelfanger, M.K., Chen, Y., Amro, E.M., Han, B., Chen, Z., Szafranski, K., Chakkarappan, S.R., Boehm, B.O., MacLean, A.L., and Rudolph, K.L. (2022). Age-dependent effects of Igf2bp2 on gene regulation, function, and aging of hematopoietic stem cells in mice. Blood *139*, 2653–2665. https://doi.org/10.1182/blood.2021012197.
20. Caiado, F., Pietras, E.M., and Manz, M.G. (2021). Inflammation as a regulator of hematopoietic stem cell function in disease, aging, and clonal selection. J. Exp. Med. *218*, e20201541. https://doi.org/10.1084/jem.20201541.
21. Itokawa, N., Oshima, M., Koide, S., Takayama, N., Kuribayashi, W., Nakajima-Takagi, Y., Aoyama, K., Yamazaki, S., Yamaguchi, K., Furukawa, Y., et al. (2022). Epigenetic traits inscribed in chromatin accessibility in aged hematopoietic stem cells. Nat. Commun. *13*, 2691. https://doi.org/10.1038/s41467-022-30440-2.
22. Caiado, F., Kovtonyuk, L.V., Gonullu, N.G., Fullin, J., Boettcher, S., and Manz, M.G. (2023). Aging drives Tet2+/- clonal hematopoiesis via IL-1 signaling. Blood *141*, 886–903. https://doi.org/10.1182/blood.2022016835.
23. Kovtonyuk, L.V., Caiado, F., Garcia-Martin, S., Manz, E.M., Helbling, P., Takizawa, H., Boettcher, S., Al-Shahrour, F., Nombela-Arrieta, C., Slack, E., and Manz, M.G. (2022). IL-1 mediates microbiome-induced inflammaging of hematopoietic stem cells in mice. Blood *139*, 44–58. https://doi.org/10.1182/blood.2021011570.
24. Wang, Y.T., Zhao, X.Y., Zhao, X.S., Xu, L.P., Zhang, X.H., Wang, Y., Liu, K.Y., Chang, Y.J., and Huang, X.J. (2015). The impact of donor characteristics on the immune cell composition of mixture allografts of granulocyte-colony-stimulating factor-mobilized marrow harvests and peripheral blood harvests. Transfusion *55*, 2874–2881. https://doi.org/10.1111/trf.13251.
25. Yamashita, M., and Passegué, E. (2019). TNF-α Coordinates Hematopoietic Stem Cell Survival and Myeloid Regeneration. Cell stem cell *25*, 357–372.e357. https://doi.org/10.1016/j.stem.2019.05.019.
26. He, H., Xu, P., Zhang, X., Liao, M., Dong, Q., Cong, T., Tang, B., Yang, X., Ye, M., Chang, Y., et al. (2020). Aging-induced IL27Ra signaling impairs hematopoietic stem cells. Blood *136*, 183–198. https://doi.org/10.1182/blood.2019003910.
27. Austin, R.J., Straube, J., Bruedigam, C., Pali, G., Jacquelin, S., Vu, T., Green, J., Gräsel, J., Lansink, L., Cooper, L., et al. (2020). Distinct effects of ruxolitinib and interferon-alpha on murine JAK2V617F myeloproliferative neoplasm hematopoietic stem cell populations. Leukemia *34*, 1075–1089. https://doi.org/10.1038/s41375-019-0638-y.
28. Zhao, K., Liu, J.F., Zhu, Y.X., Dong, X.M., Yin, R.H., Liu, X., Gao, H.Y., Xiao, F.J., Gao, R., Wang, Q., et al. (2022). Hemgn Protects Hematopoietic Stem and Progenitor Cells Against Transplantation Stress Through Negatively Regulating IFN-γ Signaling. Adv. Sci. *9*, e2103838. https://doi.org/10.1002/advs.202103838.
29. Wu, Y., Lai, X., Shi, J., Tan, Y., Zhao, Y., Yu, J., Liu, L., Zhang, W., Huang, H., and Luo, Y. (2022). Effect of donor characteristics on T cell-replete haploidentical stem cell transplantation over the last 10 years at a single institution. Br. J. Haematol. *196*, 1225–1238. https://doi.org/10.1111/bjh.17978.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Clinical data	This paper, Wu et al. 29	N/A
Raw and processed scRNA-seq data	GEO database	GSE224714
Raw and processed scRNA-seq data	GEO database	GSE104379
Raw and processed scRNA-seq data	GEO database	GSE120221
Software and algorithms		
Seurat	https://satijalab.org/seurat/index.html	Version 4.1.1
EnhancedVolcano	https://github.com/kevinblighe/EnhancedVolcano	Version 1.13.2
TBtools	https://github.com/CJ-Chen/TBtools/releases	Version 1.0
scDblFinder	https://github.com/plger/scDblFinder	N/A
SingleCellExperiment	https://bioconductor.org/packages/release/bioc/html/SingleCellExperiment.html	N/A
clusterProfiler	https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html	N/A
CellChat	https://github.com/sqjin/CellChat	Version 1.1.3
tSNE	https://github.com/jdonaldson/rtsne/	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Prof. He Huang (huanghe@zju.edu.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table.

The data and code to replicate the analysis is available at https://github.com/lixia2017/-IL-1-IL-6-TNF-Contributes-to-the-Dysfunction-and-Aging-of-HSCs.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

Data analysis of clinical data and single-cell sequencing data from allo-HSCT patients

The data of 443 patients treated with allo-HSCT were collected in a clinical trial study, which was conducted in accordance with the principles of the Declaration of Helsinki as well as with the approval of The First Affiliated Hospital of Zhejiang University. Relevant results have been published in 2021.[29]

GSE224714 was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). GSE224714 was using 10x genomics, while GSE104379 and GSE120221 were both based on C1 Single-Cell Auto Prep System. GSE224714 was obtained from 6 recipients diagnosed with aplastic anemia and treated with allo-HSCT: young donors aged 21, 23, and 15 as well as old donors aged 40, 40 and 45, accompanied by aGVHD level No, Grade I (skin II), No, Grade I (skin I), Grade IV (skin III, liver I, GI tract IV), respectively.

The processed bone marrow scRNA-seq data of patients with different degree of aGVHD after allo-HSCT from GSE224714 was chosen to represent the GVHD related bone marrow hematopoietic reconstitution with different donor ages. The patients with young donors (ages <25) and mild symptoms (No or Grade I) were defined as Young group. The patients with old donors (ages ≥40) and severe symptoms (Grade II or Grade IV) were defined as Old group. The analysis follows Seurat (version 4.1.1) pipeline. AddModule Score function and HALLMARK INFLAMMATORY RESPONSE geneset were used to evaluate the inflmmatory characteristics at single cell level.

Data filtration and integration of HSC

GSE104379 was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). In GSE104379, there were 654 HSCs from 9 healthy samples: females aged 24, 28, 36, 37, 64, and 71, and males aged 24, 66, and 71.

Quality filtration for GSE104379 was performed with R package PCA. The non-compliant data were excluded from the matrix and the removal was repeated 5 times until meeting the requirement abs (PC1, PC2) $\leq$ 75. T-distributed Stochastic Neighbor Embedding (tSNE) was also performed to visualize HSC heterogeneity among ages and between genders.

Further integration for the final qualified 624 HSCs was performed based on the package Seurat (version 4.1.1). Briefly, young (age$\leq$37) and old (age$\geq$60) data matrices were transformed into the 10X standard Seurat objects. Cell pre-processing was performed with the threshold of filtration applied to cells expressing a minimum of 200 features and a maximum of 5,000. Unsupervised dimension reduction by principal component analysis (PCA) according to variable features was performed. Subsequently, UMAP dimension reduction was performed combined with clustering, based on selected statistically significant PCs. Cluster annotation was based on the dot distribution and expression of marker genes. Feature visualization was performed with different Seurat graphs like Dimplot and DotPlot.

Differential expression and functional enrichment analysis of DEGs in HSC

To overview transcriptional differences between young and old donors, DEGs were calculated by the function FindMarkers in package Seurat. PCA plots were performed, showing the averaged position of all cells in each donor. Significantly overexpressed genes were obtained and shown via EnhancedVolcano (Version 1.13.2). Furthermore, the functional enrichment for HSC aging was assessed using enrichKEGG and GSEA in R package clusterProfiler. The heatmaps of main up-regulated and down-regulated genes in the above significant enriched pathways (p value cutoff <0.05) were shown via TBtools (version 1.0). Moreover, to probe into the features and function of each cluster, functional enrichment was performed in the same way mentioned above.

Data filtration, integration, and analysis of cells in HSC bone marrow niche

GSE120221 was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). In GSE120221, 48104 data in the bone marrow of 16 healthy samples were chosen to represent 3 age groups: young (age$\leq$31), median (31<age <60), and old (age $\geq$60).

R package Seurat (version 4.1.1) was performed for subsequent analysis, including data quality control, integration, and cluster identification. For quality control, the criteria were as follows: 1) Cells in GSE32719 were removed using scDblFinder and SingleCellExperiment package; 2) the number of genes detected in each cell was 500~2500; 3) the percentage of mitochondrial genes <10%. The filtration of the cells was performed and assessed with two metrics: the number of detected genes was above 500 and below 2500, and the percentage of mitochondrial genes was below 10. Principal component analysis was performed for 2000 variable features. UMAP was utilized for further dimension reduction, clustering, and visualization. The clusters were annotated with canonical markers. EnrichKEGG function from clusterProfiler was performed for functional pathway analysis. AddModuleScore function from Seurat and GVHD-related geneset were utilized to evaluate the GVHD score at the single cell level.

Cell-cell communication in bone marrow cells was evaluated by the R package CellChat (Version 1.1.3), which mainly depends on the datasets of ligand-receptor pairs. HSPC cells were set as the receiver in the network, while other cell types were set as the sender. Function CompareInteractions was performed for the differential and specific ligand-receptor interaction network during aging. Several functions, such as RankNet and netVisual_bubble, were used to visualize the landscape of cell-to-cell communication.

QUANTIFICATION AND STATISTICAL ANALYSIS

The optimal cut point principle and clinical reality were considered for donor age stratification (young for age$\leq$37, median for age 38–49, and old for age$\geq$50). Brown-Forsythe test was used to identify the significance of aGVHD and cGVHD levels between three age groups, while the Log Rank test was performed to demonstrate if OS distributions of young and old groups were statistically pronounced. Further correlation analysis employed the relationship between two random variables, the level of aGVHD and the OS.

A p value of less than 0.05 was considered significant. All the computational analyses were performed using the R programming environment (version 4.1.2).

ADDITIONAL RESOURCES

The clinical data in this article was approved by the the Ethics Review Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (clinical registry number: approval no. 2021IIT299, ZJU-haplo-donor01).