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Role of the bone marrow vascular niche in chemotherapy for MLL-AF9-induced acute myeloid leukemia

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Abstract

Leukemia stem cells in acute myeloid leukemia (AML) can persist within unique bone marrow niches similar to those of healthy hematopoietic stem cells and resist chemotherapy. In the context of AML, endothelial cells (ECs) are crucial components of these niches that appear to promote malignant expansion despite treatment. To better understand these interactions, we developed a real-time cell cycle-tracking mouse model of AML (Fucci-MA9) with an aim of unraveling why quiescent leukemia cells are more resistant to chemotherapy than cycling cells and proliferate during disease relapse. We found that quiescent leukemia cells were more prone to escape chemotherapy than cycling cells, leading to relapse and proliferation. Importantly, post-chemotherapy resting leukemia cells tended to localize closer to blood vessels. Mechanistically, after chemotherapy, resting leukemia cells interacted with ECs, promoting their adhesion and anti-apoptotic capacity. Further, expression analysis of ECs and leukemia cells during AML, after chemotherapy, and after relapse revealed the potential of suppressing the post-chemotherapy inflammatory response to regulate the functions of leukemia cells and ECs. These findings highlight the role of leukemia cells in evading chemotherapy by seeking refuge near blood vessels and provide important insights and directions for future AML research and treatment.

Key Words: Acute myeloid leukemia; Bone marrow vascular microenvironment; Chemotherapy; Cell cycle

1. INTRODUCTION

Hematopoietic stem cells (HSCs) reside in the bone marrow (BM) niche, where they depend on various cell types, such as endothelial cells (ECs) and perivascular mesenchymal cells, for survival and differentiation signals.[1–3] The BM niche plays a crucial role in regulating leukemia cell behavior,[4–6] implicating competition between HSCs and leukemia cells for the same niche.[7] Furthermore, leukemia cells can restructure the surrounding environment to establish a unique leukemia-permissive niche, which sustains leukemia cell survival at the expense of normal hematopoiesis.[8–10] Therefore, a prospective approach to leukemia therapy may involve targeting the dynamic communication between leukemia cells and the altered niche, as supported by several studies.[11,12]

To advance the development of targeted and well-tolerated treatments for acute myeloid leukemia (AML), it is imperative to gain insight into the mechanisms by which AML cells persist, outcompete non-malignant hematopoietic cells, and ultimately establish a favorable milieu that promotes the emergence of chemoresistant leukemia stem cells (LSCs). Perturbations of the BM niche have been reported in advanced stages of the disease.[13] Despite advances in chemotherapy, the dynamic process underlying relapse remains elusive, and further examination of this process may reveal novel therapeutic targets. AML cells secrete vascular endothelial growth factor (VEGF),[14] and the density of the BM microvasculature is increased in AML patients.[15,16] These findings highlight the importance of investigating the role of the microvasculature in AML development and progression and suggest that targeting these factors may be a promising avenue for future therapies.

The significance of leukemic-endothelial crosstalk in leukemogenesis, which involves enhanced EC growth and increased AML cell proliferation, potentially mediated by angiopoietin-1 and the STAT signaling pathway, has been demonstrated.[17] Furthermore, leukemia cells have been shown to promote the expression of adhesion factors such as Icam1, Vcam1, and E-selectin in ECs, thereby facilitating leukemia cell adhesion.[18] Our previous study revealed that interleukin 4 secreted by BM ECs contributes to thrombocytopenia in AML.[19] Moreover, we have found that angiopoietin-like 2-containing small extracellular vesicles from vascular ECs accelerate leukemia progression.[20]

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These findings underscore the importance of investigating the role of ECs in AML pathogenesis and suggest that targeting leukemic-endothelial crosstalk may be a promising therapeutic approach for AML.

LSCs are crucial in AML pathogenesis and their eradication is critical for better patient outcomes.[21] AML stem cells are chemotherapy-resistant, partially owing to their cell-cycle quiescence,[22] and tend to reside in the endosteal region of the BM. To understand how to interfere with the BM microenvironment to halt leukemia development or progression, here we developed a fluorescent ubiquitination-based cell-cycle indicator (Fucci) real-time cell cycle-tracking mouse model of AML. This model could allow us to investigate leukemic cell–microenvironment interactions during different stages of leukemia progression.

2. MATERIALS AND METHODS

2.1. Mice

Six-to-eight-week-old female C57BL/6J and B6.SJL mice were purchased from the animal facility of the State Key Laboratory of Experimental Hematology (SKLEH, Tianjin, China). All animal procedures were performed in compliance with the animal care guidelines approved by the Institutional Animal Care and Use Committees of the SKLEH and the Institute of Hematology. The procedures for the care and use of animals were approved by the Ethics Committee of the Institutional Animal Care and Use Committee of the State Key Laboratory of Experimental Hematology (approval no.: IHCAMS-DWLL-NSFC2022073-1). All applicable institutional and governmental regulations concerning the ethical use of animals were followed.

2.2. Mouse model establishment

Fucci-mixed lineage leukemia (MLL)-AF9-puro (Fucci-MA9) cells established in our laboratory were used to construct a non-irradiated AML model. The Fucci-MA9 cells were established using BM c-Kit$^+$ cells from Fucci mice, which were infected with retrovirus carrying an MSCV-MLL-AF9-IRES vector and collected 48 hours after infection. For retrovirus production, 7 μg of the retroviral plasmid, 5 μg of pKat, and 3 μg of pVSVG were transfected into 293T cells using Lipofectamine 2000. After 48 and 72 hours of culture, supernatants were harvested and concentrated using an Amicon filter. To establish the non-irradiated mouse model, 1×10^6 spleen cells (CD45.2$^+$) harvested from primary Fucci-MA9 leukemic mice were injected into non-irradiated recipients (CD45.1$^+$).

2.3. Immunofluorescence

VE-cadherin-APC antibody (10 μg, dissolved in 300 μL of phosphate-buffered saline [PBS]) was injected into the tail vein of diseased Fucci-MA9 mice. After 10 minutes, the mice were anesthetized. The femurs were harvested using scissors and forceps and fixated in 4% paraformaldehyde overnight. Then, they were embedded in optimal cutting temperature compound and cut into 2 halves. The half bones were transferred into 0.6-mL Eppendorf tubes, and 0.4 mL of staining buffer (PBS + 10% dimethyl sulfoxide + 0.5% IPGEAL + 5% donkey serum) and 4′,6 diamidino-2-phenylindole (DAPI; Sigma-Aldrich) were added. The samples were incubated on a shaker protected from light at room temperature for 4 hours. Then, they were washed with PBS for 2 hours and scanned using a 2-photon laser confocal microscope.

2.4. Flow cytometry

All antibodies used were obtained from BD Biosciences or e-Bioscience. Cells were stained with DAPI to exclude dead cells. For flow cytometry, we used antibodies for lineage cells (Gr-1-APC-CY7, CD3-APC-CY7, CD8-APC-CY7, Mac-1-APCCY7, CD4-APC-CY7, Ter119-APC-CY7, and B220-APC-CY7), LSCs (Sca-1-PE-CY7, c-Kit-BV605, CD16/32-PerCP-CY5.5, and CD34-FITC), and leukemia cells (CD45.1-Percp-CY5.5 and CD45.2-PE-CY7).

2.5. Flow-cytometric analysis of mouse BM cells

BM cells were flushed from mouse hindlimb bones into a 5-mL flow cytometry tube using a 1-mL syringe and centrifuged at 377×*g* for 5 minutes. The supernatant was discarded and the cells were resuspended in 1 mL of PBS containing 2% fetal bovine serum (FBS). A number of cell-labeling antibodies were added to each sample and the cells were incubated at 4°C for 30 minutes. The cells were washed with 1 mL of PBS to remove non-specifically bound antibody and resuspended in 300 to 500 μL of PBS containing 2% FBS. Finally, the cells were analyzed by flow cytometry.

2.6. Statistical analysis

The GraphPad Prism 5.0 software was used for statistical analysis. Unpaired Student *t* tests and analysis of variance were used to generate *P* values for most of the datasets. Differences at $P < .001$ were considered significant.

3. RESULTS

3.1. Visualization of the cell cycle in leukemia cells

The inherent resistance of LSCs to anti-proliferative therapies underscores the need to identify potential therapeutic targets by accurately tracing and clarifying the location and heterogeneity of LSCs. A previous study revealed that AML stem cells tend to reside in the G_0 phase of the cell cycle, which contributes to their resistance to chemotherapy.[22] To validate the quiescent nature of LSCs, we subjected LSCs to analysis. We observed that 42.33% ± 1.52% L-GMP cells were in the G_0 phase and L-CD34$^{+/-}$ LKS cells predominantly resided in the G_0/G_1 phase (63.67% ± 2.33%), confirming the cell-cycle quiescence of LSCs. To enable real-time monitoring and analysis of the LSC distribution, we established a Fucci mouse model, termed Fucci-MA9, by infecting c-Kit$^+$ cells with MLL-AF9 (MA9) retrovirus. This model allows for the visualization of the cell-cycle phase and spatial distribution of leukemia cells (Fig. 1A).

The Fucci technology, which utilizes 2 antiphase oscillating proteins, enables the visualization of natural cell-cycle progression in living cells in vivo and in vitro. By employing Cdt1-labeled with monomeric Kusabira-Orange 2 (Cdt1-mKO2) and geminin labeled with monomeric Azami-Green (Gem-mAG) as reporters, which are controlled by the cell-cycle machinery through ubiquitin-mediated proteasomal degradation, we were able to visualize the cell-cycle phase of leukemia cells.[23] Cdt1-mKO2 is expressed in cells in the G_0/G_1 phase and detected by red fluorescence, whereas Gem-mAG is expressed in cells in the $S/G_2/M$ phase and detected by green fluorescence (Fig. 1B, D). In the Fucci-MA9 model, 51.21% ± 4.97% of leukemia cells were in the G_1/S phase, whereas the $S/G_2/M$ phase accounted for 24.97% ± 3.23% and the G_0/G_1 phase only for 5.5% ± 0.4% of leukemia cells (Fig. 1C). The specificity and accuracy of the model were verified by an EdU incorporation assay (Fig. 1E).

3.2. The BM vascular microenvironment is remodeled during AML progression

To investigate the mechanism by which resting leukemia cells survive chemotherapy and evolve into therapy-resistant clones, we subjected Fucci-MA9 model mice to chemotherapy treatment. Peripheral blood leukemia cells were monitored and when their

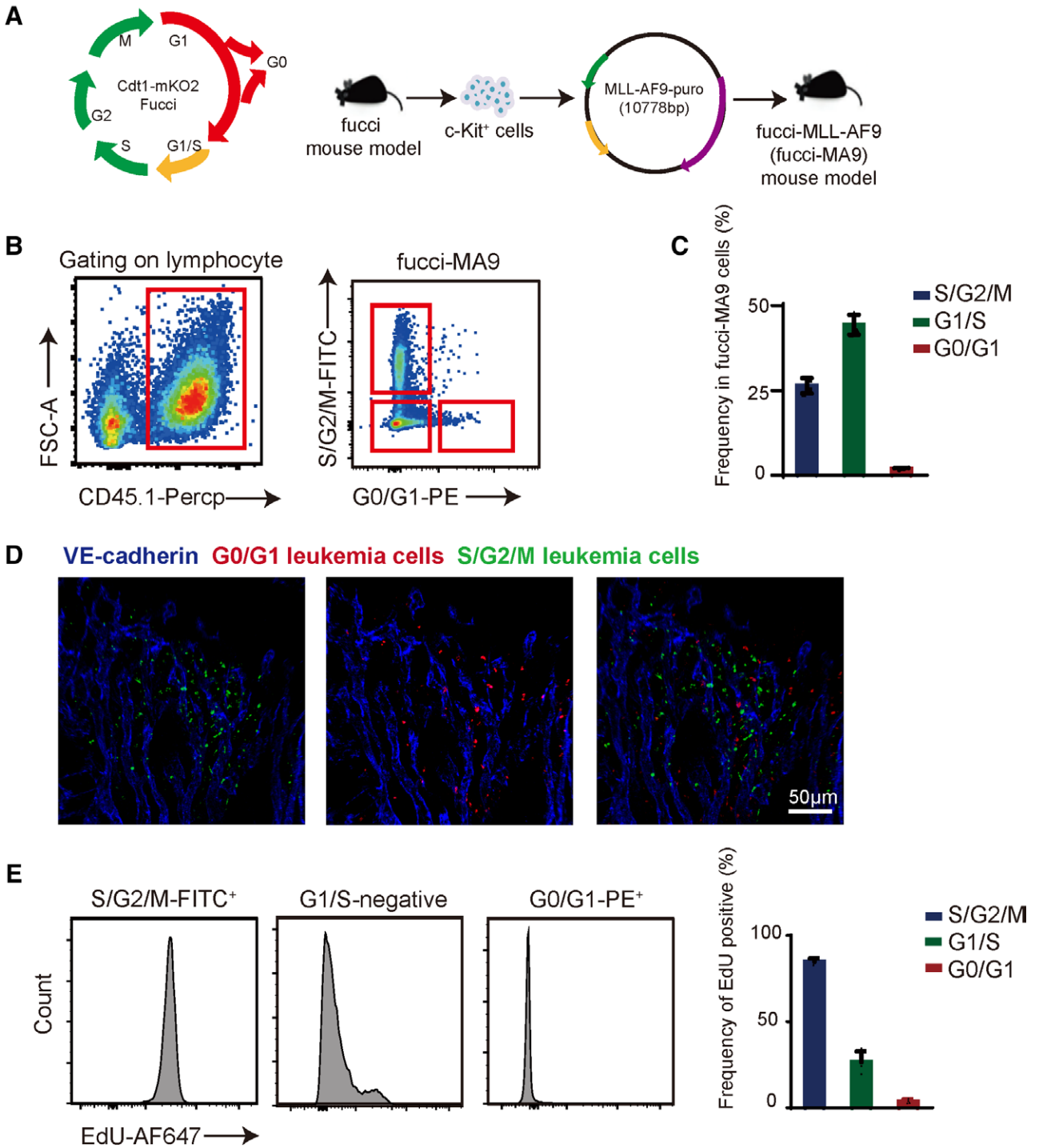

Figure 1. Visualization of the cell cycle in leukemia cells. (A) Schematic representation of the establishment of a Fucci-MA9 mouse model. (B) In the late stage of AML (leukemia cells accounting for 80%–90% of BM cells), flow-cytometric analysis of Fucci AML cells was conducted (left panel), and the distribution of leukemia cells in the indicated cell-cycle phases was determined (right panel). (C) Histogram showing the frequency of Fucci-MA9 cells in different cell-cycle phases (n = 4). (D) Tile scan image showing Fucci-MA9 cells in the BM. (E) Flow-cytometric analysis of EdU incorporation by leukemia cells in different cell-cycle phases. The histogram shows the frequency of EdU-positive cells (n = 4). AML = acute myeloid leukemia, BM = bone marrow.

proportion reached 5%, combination therapy with cytarabine (100 mg/kg) and daunorubicin (3 mg/kg) was administered for 5 days, resulting in a significant decrease in leukemia cells (peripheral blood leukemia cells <5%). Following drug withdrawal, peripheral blood was again continuously monitored until the proportion of leukemia cells reached >5%, indicating relapse (Fig. 2A). The mean pre-treatment AML engraftment, measured as the percentage of peripheral blood CD45.1$^+$ cells, was similar between chemotherapy-treated and untreated mice (4.9% ± 0.2% and 4.6% ± 1.1%, respectively, P = .841). After chemotherapy, leukemia cells in the S/G_2/M phase were significantly reduced (0.51% ± 0.1% in the treatment group vs 24.97% ± 3.23% in the control group, P < .0001). In contrast, quiescent leukemia cells increased drastically after chemotherapy (54.1% ± 2.3% in the treatment group vs 5.5% ± 0.4% in the control group, P < .0001) (Fig. 2B). These results suggested that quiescent leukemia cells exhibit chemotherapy resistance.

To comprehensively analyze the distribution of resting leukemia cells, we utilized Fucci-MA9 mice to track the dynamic

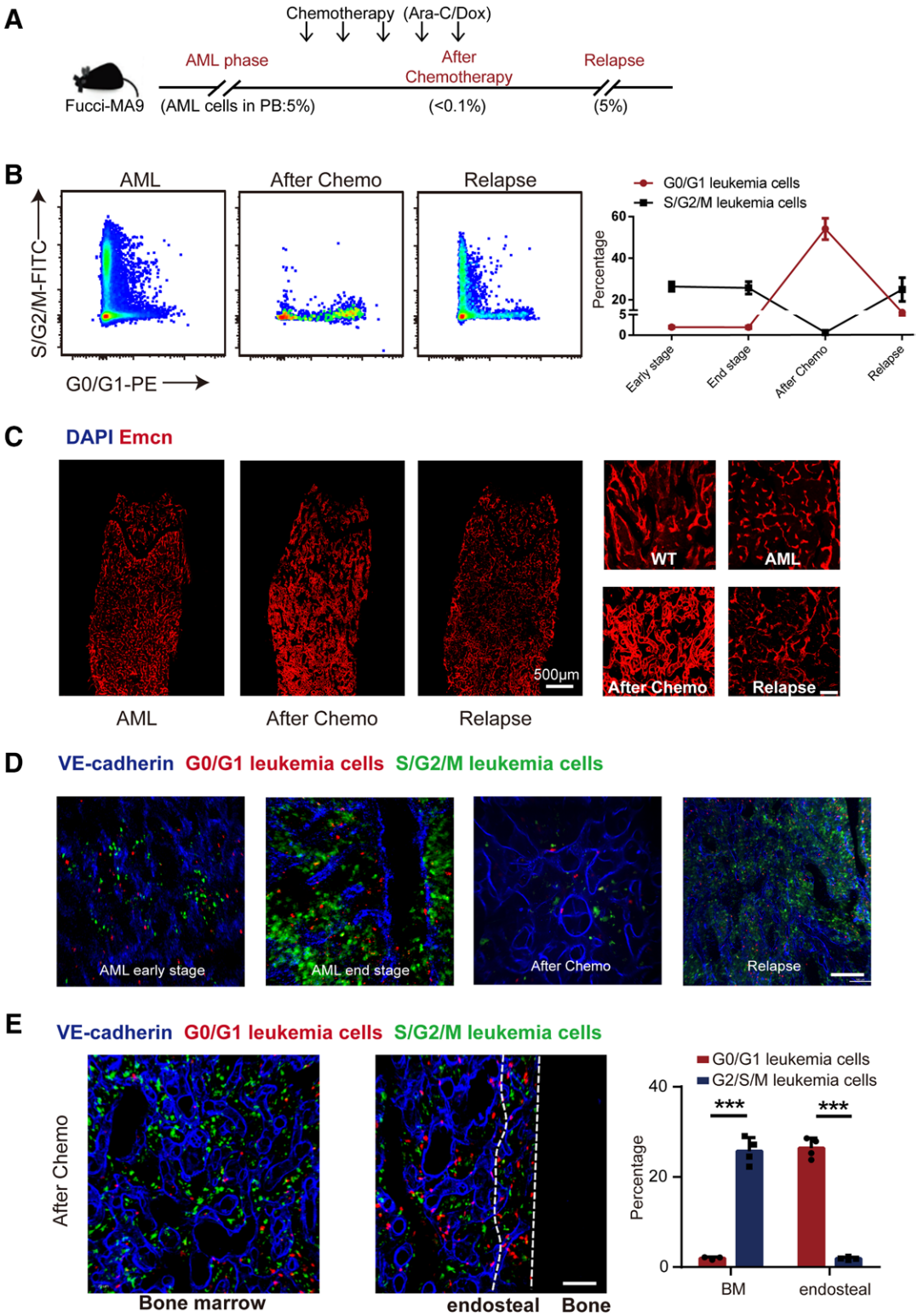

Figure 2. The BM vascular microenvironment is remodeled during AML development. (A) Schematic representation of the chemotherapy regimen used in Fucci-MA9 mice. (B) Flow-cytometric analysis of Fucci AML cells collected during leukemia (left panel), after chemotherapy (central panel), and after relapse (right panel). The curves show the alterations in the percentages of Fucci-MA9 cells in different cell-cycle phases. (C) Tile scans of bone metaphysis and diaphysis showing vessels in AML, after chemotherapy, and after relapse. The right panels show details of the vessels (scale bar: 50 μm). (D) Representative images showing blood vessels and Fucci-MA9 cells in the BM (scale bar: 50 μm). (E) Representative images of the central marrow and endosteal region showing blood vessels and Fucci-MA9 cells (scale bar: 50 μm). The right panel shows the percentages of Fucci-MA9 cells in different cell-cycle phases in the central marrow and endosteal region (n = 4). AML = acute myeloid leukemia, BM = bone marrow, DAPI = 4′,6 diamidino-2-phenylindole.

cell-cycle progression of leukemia cells and examined their function in different phases, including active leukemia, after chemotherapy, and after relapse. Four time points were selected for this analysis: early-stage leukemia (leukemia cells accounting for 10%–20% of BM cells), end-stage leukemia (leukemia cells accounting for 80%–90% of BM cells), after chemotherapy, and

after relapse. During leukemia development, cells were predominantly in the proliferative (S/G2/M) phase (Fig. 2B). However, following chemotherapy treatment, cycling leukemia cells were significantly reduced in number, and the remaining leukemia cells were mostly in the resting phase. On relapse, approximately 90% of resting leukemia cells re-entered the cell cycle.

In vitro coculture of ECs with leukemia cells promotes leukemia cell proliferation.[17] Furthermore, ECs activated by VEGF-A facilitate leukemia progression and promote adhesion between leukemia cells and ECs,[24] and VEGF-C, which is secreted by ECs, regulates leukemia cell survival and proliferation via VEGFR-3-mediated signaling.[25] These findings suggest that interactions between ECs and leukemia cells may play a crucial role in promoting leukemia cell survival and proliferation.

Immunostaining of ECs revealed that endosteal AML cells gradually degraded the endosteal endothelium in leukemia, whereas the central marrow remained vascularized, with an increased number of sinusoidal vessels. However, after chemotherapy treatment, blood vessels expanded to a round shape with a destroyed structure. Notably, after relapse, the expanded round vessels disappeared and the vascular structure returned to sinusoidal vessels, like in leukemia (Fig. 2C, D). These findings indicated that the BM vascular microenvironment constantly changes during leukemia development and may play a role in the disease process. Analysis of the overall distribution of leukemia cells after chemotherapy revealed that quiescent leukemia cells preferentially migrated to the endosteal region (34.8% ± 2.2% in the endosteal, 6.6% ± 1.3% in the BM cavity), whereas cycling AML cells were primarily concentrated in the center of the BM cavity (4.9% ± 1.6% in the endosteal area, 36.5% ± 1.7% in the BM cavity) (Fig. 2E). These results suggested that during chemotherapy, leukemia cells may migrate to and remodel the surrounding vascular microenvironment, potentially contributing to the survival of leukemia cells.

3.3. Resting leukemia cells are located closer to blood vessels after chemotherapy

To analyze the location of AML cells in different cell-cycle phases in more detail, we performed a 3-dimensional reconstruction of the bone section, which revealed that 37.18% ± 3.52% of leukemia cells were concentrated within 0 to 4 μm of blood vessels, in contrast to nucleated cells (2.45% ± 0.36%, $P < .001$) (Fig. 3A). This result corroborated the importance of the BM vascular microenvironment in the regulation of leukemia cell survival and progression.

After chemotherapy, the distance between leukemia cells in the resting phase and blood vessels was shorter than that between cells in the cycling phase and blood vessels (Fig. 3B, D). Particularly, 46.18% ± 5.51% of cells in resting phase and 18.59% ± 1.43% of cells in cycling phase were observed within 0 to 4 μm from blood vessels ($P < .001$). These observations, together with the above flow-cytometric analysis results, suggested that during chemotherapy, resting leukemia cells appear to make cell–cell contact with ECs. Interestingly, we found that during relapse, quiescent LSCs no longer aggregated with ECs. Instead, both proliferating and quiescent cells were scattered around the blood vessels (Fig. 3C). These findings suggested that chemotherapy may prompt perivascular migration of resting leukemia cells. The data presented here provide insights into the dynamic interplay between leukemia cells and the BM vascular microenvironment during chemotherapy treatment and relapse.

To assess the impact of chemotherapy on leukemia cells in different cell-cycle stages, we examined the apoptosis of leukemia cells. Interestingly, the rate of apoptosis of $S/G_2/M$ leukemia cells significantly increased after chemotherapy, whereas that of G_0/G_1 leukemia cells did not (Fig. 3E). The proportion of apoptotic $S/G_2/M$ leukemia cells was significantly higher than that of G_0/G_1 leukemia cells after chemotherapy. These findings suggested that chemotherapy may prompt resting leukemia cells to migrate around blood vessels to resist chemotherapy damage.

3.4. Transcriptomic characteristics of ECs and resting leukemia cells

Previous studies, including our own, have highlighted the crucial role of ECs in AML development.[19,20,26] Our current findings demonstrated that ECs also contribute to the resistance of leukemia cells to chemotherapy, likely by interacting with resting leukemia cells. This finding prompted us to investigate how the vascular niche may contribute to the chemoresistance of LSCs. To this end, we conducted RNA-sequencing of both ECs and resting leukemia cells in different phases, including in leukemia, after chemotherapy treatment, and after relapse.

Cluster analysis revealed significant differences between the transcriptome of ECs after chemotherapy and those of ECs in leukemia and after relapse. Moreover, chemotherapy altered the transcriptional characteristics of quiescent leukemia cells. Interestingly, ECs and leukemia cells had a high degree of similarity in leukemia and after relapse (Fig. 4A). Moreover, we found significant changes in the endothelial transcriptome after chemotherapy, indicative of dynamic changes consistent with treatment-induced structural alterations in the bone vasculature.

Gene Ontology (GO) analysis revealed that after chemotherapy, the most significantly enriched pathways in both ECs and quiescent leukemia cells as compared with the leukemia phase were related to migration, interaction, adhesion, and inflammation signaling (Fig. 4B). The inflammatory response post-chemotherapy may facilitate the movement of leukemia cells toward the perivascular site of proliferation. The RNA-sequencing data indicated that ECs may express inflammatory and adhesion factors following chemotherapy, leading to the proximity of resting leukemia cells to vascular ECs, enabling them to evade chemotherapy.

We hypothesized that the adhesion of leukemia cells to ECs is attributed to the enhanced interaction with ECs. To confirm this hypothesis, we performed protein–protein interaction enrichment analysis between ECs and resting leukemia cells after chemotherapy versus in AML. As expected, various genes involved in leukocyte migration, cell adhesion, and extracellular matrix organization at the vascular wall (Fig. 4C) were upregulated in ECs and leukemia cells during chemotherapy versus in AML, suggesting that ECs and the extracellular matrix contribute to the adhesion of leukemia cells. This finding indicated the potential impact of chemotherapy on the molecular mechanisms underlying the interaction between the 2 cell types, ultimately affecting AML progression and treatment outcomes.

In line with our earlier observations, we found that the expression levels of numerous mRNAs associated with the development of leukemia, including *Dusp6*,[27] *Klf4*,[28] and *Plxnb2*,[29] were significantly elevated in ECs and resting leukemia cells after chemotherapy (Fig. 4D). These findings suggested that ECs may play a crucial role in promoting leukemia cell growth after chemotherapy. Interestingly, we found that the ECs expressed certain chemotaxis-associated genes, such as *Cxcl12*, which may further facilitate leukemia cell migration and proliferation.[30] Furthermore, the expression of inflammation- and leukemogenesis-related genes, such as *Chil1*,[31–33] *Atf3*,[34] and *Itgb2*,[35] was upregulated in quiescent leukemia cells after chemotherapy (Fig. 4D). These results suggested that AML-induced BM vascular remodeling may be more complex than simply the induction of angiogenesis and that subtle and progressive changes may significantly affect the ecological competition between leukemia and healthy

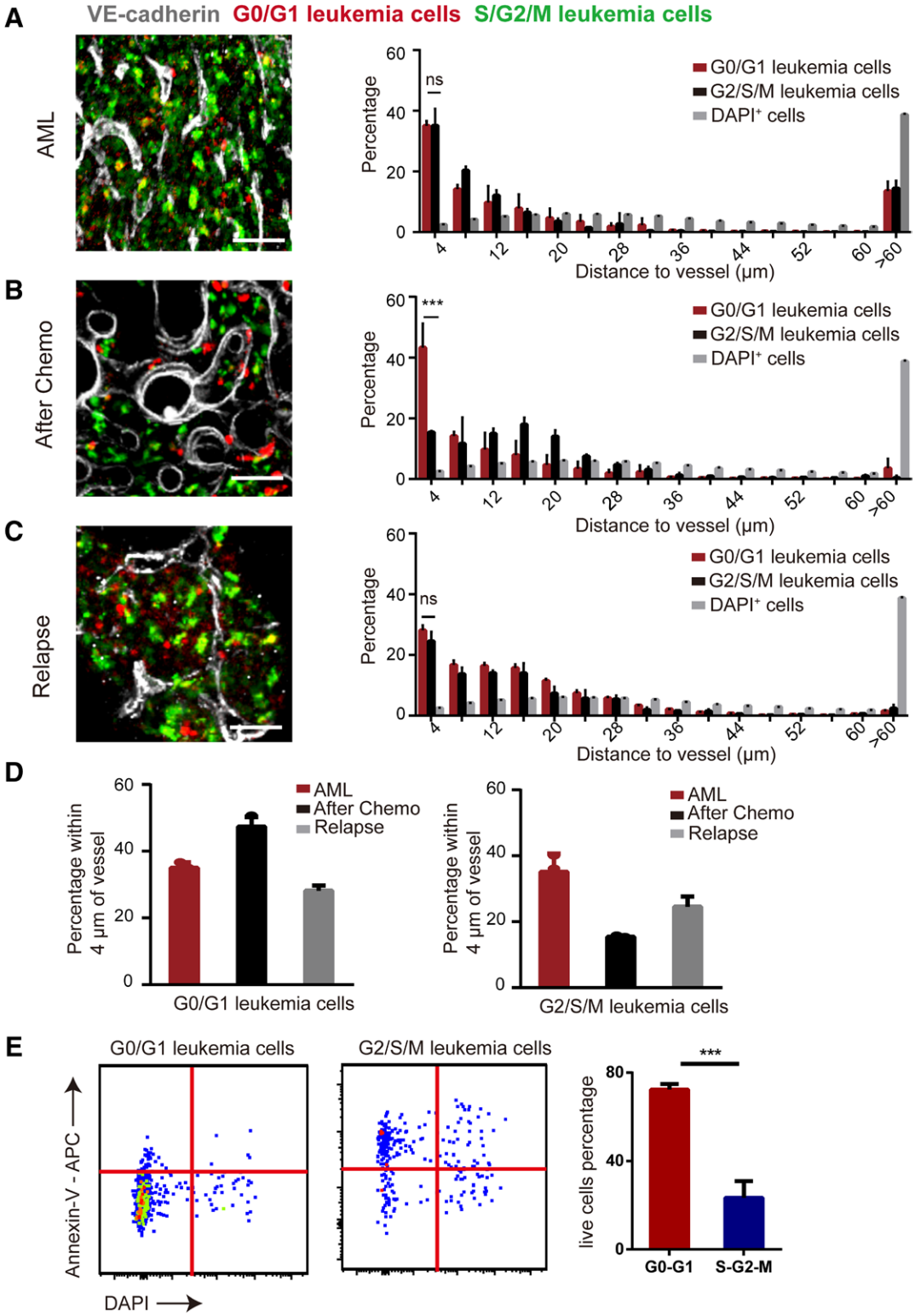

Figure 3. Resting leukemia cells are located closer to blood vessels after chemotherapy. (A–C) Representative images showing blood vessels and Fucci-MA9 cells in the BM in the 3 phases examined (scale bar: 50 μm). The right panels show the distance of leukemia cells or nucleated cells to the nearest ECs (5000–6000 leukemia cells were counted in the BM from 4 femurs). (D) Percentages of leukemia cells in different cell-cycle phases within 4 μm of the blood vessel (n = 4). (E) Flow-cytometric analysis of the apoptosis of Fucci AML cells (left panel). The histograms show the percentages of live leukemia cells in different cell-cycle phases after chemotherapy (right panel) (n = 4). AML = acute myeloid leukemia, BM = bone marrow, DAPI = 4′,6 diamidino-2-phenylindole.

hematopoiesis. Indeed, these nuanced changes may contribute to the complex interplay between leukemia cells and the vascular microenvironment, ultimately determining the fate of the disease.

4. DISCUSSION

Chemotherapy is the standard treatment for patients with leukemia; however, its effectiveness is limited due to the emergence of chemoresistant cells. Recent studies have

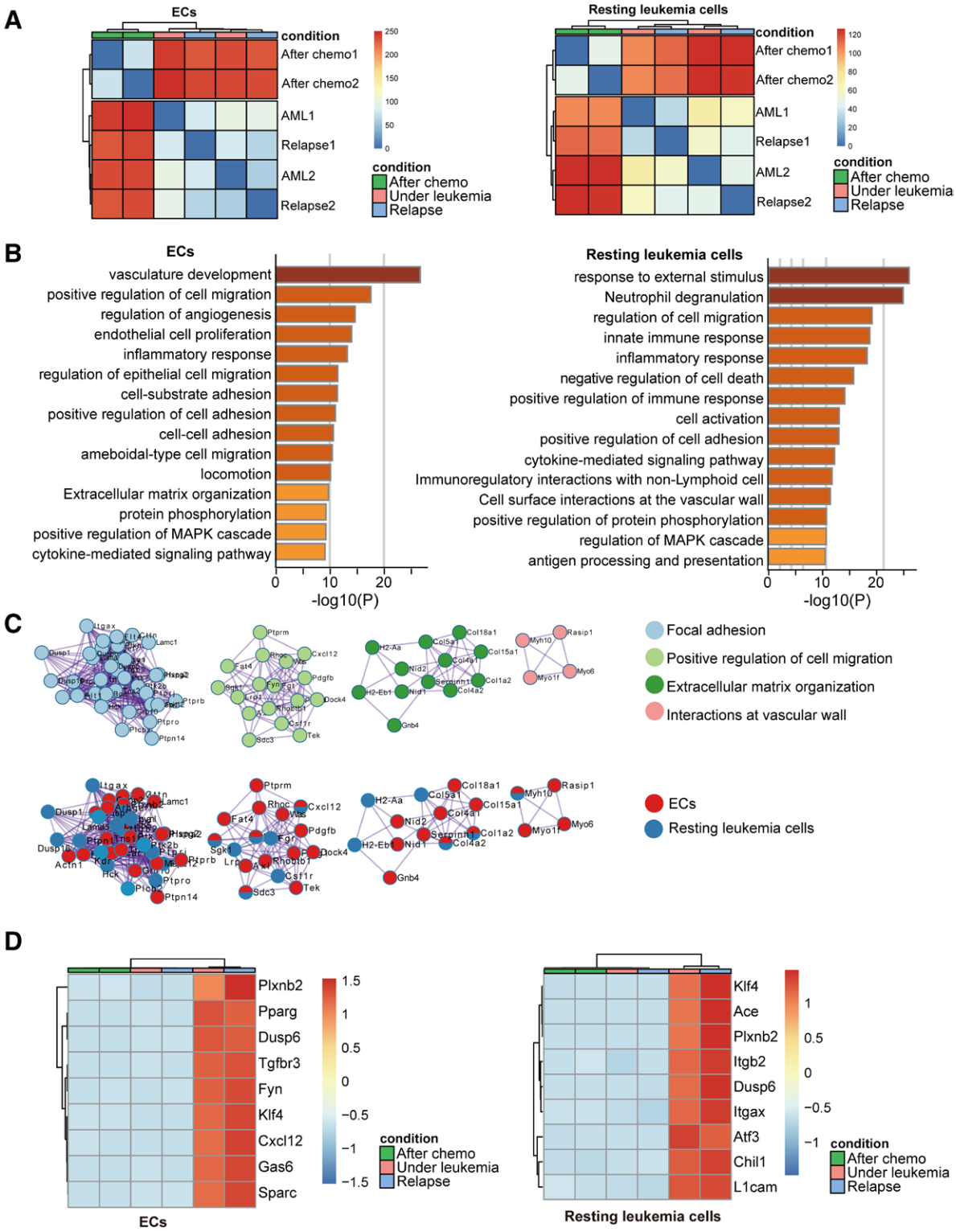

Figure 4. Transcriptomic characteristics of ECs and resting leukemia cells. (A) Cluster analysis of ECs and resting leukemia cells in AML, after chemotherapy, and after relapse. Right panel: ECs; left panel: resting leukemia cells. (B) GO term enrichment in ECs and resting leukemia cells after chemotherapy versus in AML. Right panel: ECs; left panel: resting leukemia cells. (C) Protein–protein interaction enrichment analysis of differentially expressed genes in ECs and quiescent leukemia cells. Data were downloaded from the following databases: STRING8, BioGrid9, OmniPath10, and InWeb_IM11. The network consisted of a protein subset formed by interacting proteins in ECs and quiescent leukemia cells and was analyzed using algorithms to identify enriched pathways and sources. (D) Heatmaps showing the nine most differentially expressed genes in resting leukemia cells and ECs after chemotherapy versus in AML. AML = acute myeloid leukemia, EC = endothelial cell, GO = Gene Ontology.

highlighted the critical role of the BM vascular microenvironment in the development of chemotherapy resistance.[15,24] The vascular niche provides a protective environment for leukemia cells, enabling them to evade chemotherapy-induced damage. In particular, ECs play a crucial role in regulating the interactions between leukemia cells and their protective

microenvironment. The remodeling of the BM vasculature and the transformation of HSC niches into a leukemia microenvironment following chemotherapy further exacerbate the limitations of chemotherapy. On chemotherapy, quiescent leukemia cells tend to migrate toward ECs, which express inflammatory and adhesion factors that may contribute to the chemoresistance of AML stem cells. In summary, the BM vascular microenvironment plays a significant role in the development of chemotherapy resistance in leukemia. Therefore, understanding the interactions between leukemia cells and their protective microenvironment is crucial for developing effective therapeutic strategies to overcome chemotherapy resistance and eradicate leukemia.

Our study provided significant insights into how AML cells and chemotherapy altered the BM vasculature and transformed HSC niches into a leukemia microenvironment. The Fucci-MA9 mouse model used in our study allowed us to comprehensively track the cell-cycle dynamics of leukemia cells and examine their functions in different phases, including in leukemia, after chemotherapy, and after relapse. Our findings revealed that following chemotherapy, most remaining leukemia cells entered a resting phase and only re-entered the cell cycle upon relapse, leading to significant expansion. Therefore, to achieve leukemia eradication, understanding the interactions between quiescent LSCs and their protective vascular microenvironment is crucial. In this regard, our analysis of the distribution and characteristics of resting leukemia cells and ECs provided a fascinating insight. We observed that on chemotherapy, quiescent leukemia cells were closer to blood vessels, indicating that they interacted with ECs, which may have contributed to the chemoresistance of AML stem cells. Furthermore, RNA-sequencing of resting leukemia cells and ECs indicated enhanced cell-to-cell interactions after chemotherapy versus in primary leukemia. GO enrichment analysis of ECs and quiescent leukemia cells revealed inflammation, adhesion, and cell activation signaling in both cell types. GO network analysis suggested that inflammation-related pathways play a bridging role in vascular development and leukocyte adhesion. We further speculated that ECs may express inflammatory and adhesion factors after chemotherapy, stimulating the migration of resting leukemia cells to vascular niches and promoting chemoresistance.

In conclusion, our study highlighted the potential of suppressing the inflammatory response after chemotherapy to regulate the functions of leukemia cells and ECs, providing new therapeutic directions for preventing chemotherapy resistance. In particular, inhibiting the adhesion of resting leukemia cells to ECs and modulating the inflammatory response in the vascular microenvironment may be promising strategies to this end. Our data showed that ECs could push resting leukemia cells toward perivascular niches, enabling them to evade chemotherapy damage. By understanding the interactions between leukemia cells and their protective vascular microenvironment, we can develop better strategies to overcome chemotherapy resistance and eradicate leukemia.

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AUTHOR CONTRIBUTIONS

Concept and design: H.C. and T.C.; data collection and analysis: C.X., T.L., and X.L., drafting of the article: C.X. and H.C.; critical revision of the article for important intellectual content: H.C., study supervision: H.C. All the authors approved the final article.

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