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Clustered immature myeloid precursors in intertrabecular region during remission evolve from leukemia stem cell near endosteum and contribute to disease relapse in acute myeloid leukemia

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ABSTRACT

Most acute myeloid leukemia (AML) cannot be cured because leukemia stem cells (LSC) will contribute to eventual relapse. However, how LSC initiate relapse is not yet fully understood. We performed a retrospective study on bone marrow sections from AML patients during complete remission (CR), demonstrating that single and double immature myeloid precursors were located near endosteum and clustered precursors ($\geqslant$3 cells/group) in intertrabecular region. Based on our observations, we hypothesize that after retrieval of myelotoxic regimen, LSC harboring near endosteum divide and differentiate into progeny cells which proliferate and then form colony clusters. Meanwhile, these clusters may migrate to intertrabecular region under the actions of cell migration factors. Without any interventions, clustered immature myeloid precursors may proliferate and hemotologic relapse is then unavoidable.

Introduction

Leukemia stem cells (LSC) are a population of leukemic cells possessing the potential to differentiate into hierarchical leukemic blasts. They are able to duplicate themselves and present resistance to treatments [1]. In a xenograft model, it was observed that LSC were resistant to cytarabine and were prominently located near the endosteal surface of bones after chemotherapy [2]. Consequently, it is thought that chemo-resistant LSC are responsible for leukemia relapse. However, how these LSC cause leukemia relapse is not fully understood. In some acute myeloid leukemia (AML) patients in complete remission (CR), we observed clusters of immature myeloid precursors in bone marrow sections and these clusters heralded early relapse in these patients. We speculate that this may explain part of the relationship between LSC presenting in trabecular bone, clusters, and disease relapse. Histological examination of bone marrow sections from AML patients in CR may be used to understand how LSC initiate disease relapse.

As shown in Fig. 1a, using computer image processing technology [3], we observed that single and double immature myeloid precursors were located abutting endosteum followed by clusters of precursor cells located in the intertrabecular region in some AML patients in CR. Since these clusters are both morphologically and anatomically similar to abnormal localization of immature precursors (ALIP) in myelodysplasia syndromes (MDS), they were defined as ALIP-like clusters in this paper. Additionally, we observed a similar distribution of single and double immature myeloid precursors in sections from healthy individuals. However, there were no clusters of >3 precursors in healthy human bone marrow sections (Fig. 1b). Fig. 1c and d shows that ALIP-like clusters contain CD68- and CD117-positive immature myeloid precursor cells.

Retrospective studies showed that all high-risk MDS patients were ALIP-positive and that their delay before transforming to AML was much shorter, compared with ALIP-negative MDS patients [4,5]. These reports indicated that ALIP predicted a poor prognosis in MDS. We observed that among 13 AML patients in CR without ALIP-like clusters, only 2 cases relapsed during our follow-up. However, among 49 cases with ALIP-like clusters in CR, 27 cases relapsed ($P = 0.025$) (Table 1). In addition, the presence of ALIP-like clusters in AML patients in CR indicated shorter relapse-free survival (unpublished data). Thus, both MDS and AML seem to share similar underlying mechanisms involving the formation of ALIP and ALIP-like clusters. However, mechanisms leading to ALIP-like clusters formation in AML patients in CR are still not fully understood.

Hypothesis

ALIP-like clusters in AML patients in CR originate from LSC hiding near the endosteum (or endosteal niche) during chemotherapy

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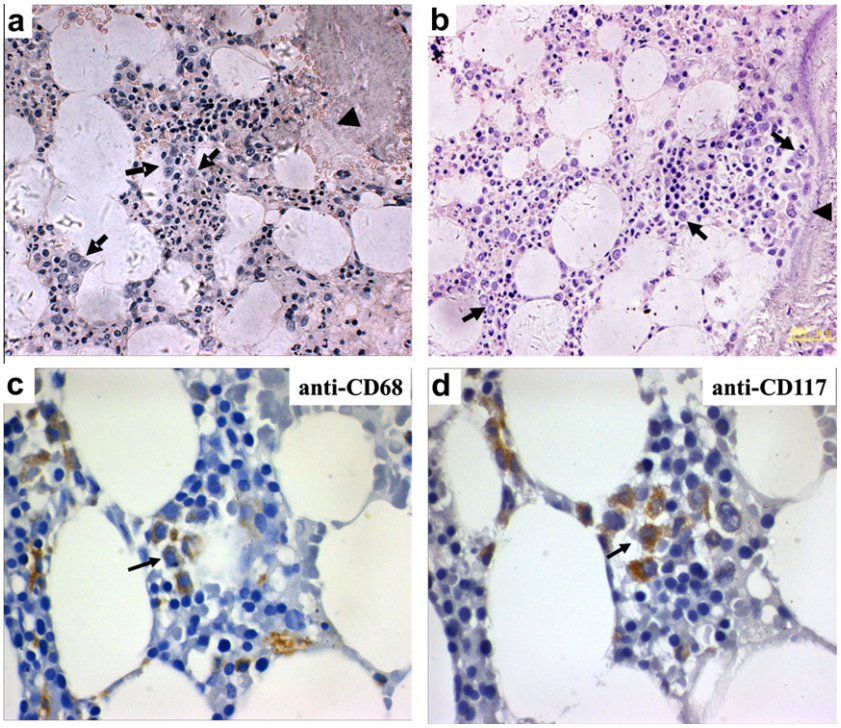

Fig. 1. Precursors distribution in acute myeloid leukemia (AML) patients in complete remission (CR) and in healthy human. (a) Bone marrow biopsy section from a patient with AML in CR showed single precursor located near trabecular bone followed by 2-cells cluster and then by abnormal localization of immature precursors (ALIP)-like cluster. (b) Bone marrow section from healthy human showed localization of precursors is similar with CR patients. However, no ALIP-like precursors were found in healthy human's bone marrow sections. Arrows indicate precursors; arrowhead indicates trabecular bone (haematoxilin giemsa acid fuchsin, 400×). (c) and (d) showed ALIP-like clusters contain immature myeloid precursors which were both CD68 and CD117 positive, while immunostaining was performed. Arrows show an ALIP-like cluster (1000×).

but not from residual in situ leukemic cells. After chemotherapy withdrawal, LSC divide and differentiate into their progeny cells, which are observed as precursor cells in bone marrow sections and are located near the endosteum. Additionally, the precursors near trabecular bone area are functionally more potent than those in intertrabecular area, although all precursors are morphologically homogeneous leukemic cells.

During their differentiation, the progeny cells/precursors simultaneously migrate to the intertrabecular region (equivalent to vascular niche) with bidirectional cytokines relations between precursors and their microenvironment. For example, leukemic cells may migrate to endothelial cells through the VEGF/VEGFR interaction. Meanwhile, exposure of the bone marrow microenvironment to increased VEGF levels may also contribute to leukemia expansion via paracrine or autocrine stimulation of subsets of leukemic cells [6]. The stromal cell-derived factor-1 (SDF-1) is a chemotactic chemokine that signals through the CXC chemokine receptor 4 (CXCR4), which is expressed by malignant hematopoietic cells. The SDF-1/CXCR4 axis is involved in the migration process of leukemic cells [7–9]. AML cells also express CD31 and CD38, the molecules could interact with the microenvironment (CD31/CD31 and CD38/CD31 pathways) and hyaluronate (CD38/hyaluronate pathway). Therefore, CD31/CD38 expression on leukemic cells may be also important for leukemic cells trafficking in AML [10]. Finally, the interaction between intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-1 (LFA-1) also play important roles in migration of AML cells [11]. Concurrently, cytokines, including some autocrine factors, may contribute to immature myeloid precursors' proliferation and clonogenicity during their migration to the intertrabecular area. In this setting, ALIP-like clusters appear. The progression to ALIP-like clusters formation is illustrated in Fig. 2. Without any administration, ALIP-like precursors should proliferate and eventual hematologic relapse is unavoidable.

Materials and methods

Histopathology

Bone marrow biopsy samples were collected from AML patients during CR and fixed in Bouin fixative (75% picric acid, 20% formalin, 5% acetic acid). Dehydration was performed using an increasing ethanol concentration gradient. All samples were embedded in Hemapun 865 plastic. Bone marrow sections of 3 μm were stained with hematoxilin-giemsa-acid fuchsin (HGF) for morphological analysis. For immunohistochemical analysis, bone marrow sections of 3 μm were treated using a microwave epitope retrieval technique with citrate buffer pH 6.0 in 90 °C for 15 m and were incubated with mouse anti-human/mouse CD68 monoclonal antibody (ab955, Abcam, Cambridge, UK) and rabbit anti-human CD117 polyclonal antibody (A4502, DAKO, CA, USA) overnight at 4 °C. Staining was performed with UltraVision Quanto detection system HRP polymer (TL-060-QHD, Thermo Scientific, CA, USA) and with 3,3′-diaminobenzidine (DAB) substrate-chromogen (Thermo Scientific). Specimens were observed using an optical microscope imaging system (Olympus, Tokyo, Japan).

Computer-processing image technology

To detect the distance from precursors to trabecular, computer-assisted image processing was performed, according to a previously published method [3]. Otsu's method was first used to automatically create a histogram shape-based image threshold, as well as to transform the gray tones image to a binary image. Otsu's method assumes that the image contains two classes of pixels (e.g. foreground and background); it then calculates the optimum threshold separating those two classes so that their combined spread (intra-class variance) would be minimal. The distance