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Leukemia Propagating Cells Rebuild an Evolving Niche in Response to Therapy

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SUMMARY

Residence of cancer-propagating cells (CPCs) within preferential microenvironmental niches has a major part in evading therapy. However, the nature of niches involved and the mechanisms protecting CPCs remain largely unknown. We addressed these issues in mouse transplantation models of acute lymphoblastic leukemia (ALL). When the engrafted leukemic cells substantially damaged adjacent microenvironment in the bone marrow (BM), after chemotherapy small foci of CPCs were retained, surrounded by sheaths of supporting cells that comprise a protective niche. We investigated patients' BM biopsies and found evidence of a similar process in patients receiving induction therapy. The efficacy of chemotherapy was enhanced by interfering with the niche formation or function. We therefore identified a therapy-induced niche that protects CPCs.

INTRODUCTION

Surviving cancer-propagating cells (CPCs) after therapy cause relapses. Studies on this issue have focused on cell-intrinsic aspects, including the importance of differentiation stage or epigenetic programs in resistant subpopulations, or the importance of genetic variegation among different subclones in an individual cancer (Anderson et al., 2011; Ding et al., 2012; Hong et al., 2008; Kreso et al., 2013; Notta et al., 2011; Sharma et al., 2010). However, evidence has suggested that subclonal residence of CPCs within preferential microenvironmental niches may have a major part in evading therapy, but this issue should be further investigated in terms of the nature of niches involved and the mechanisms that protect surviving subclones (Lane et al., 2009; Meads et al., 2009).

Recent advances in the knowledge of normal hematopoietic stem cell (HSC) niches and technologies applied to investigate such niches have allowed us to investigate these issues in the context of leukemia in the bone marrow (BM). HSCs are thought to reside in at least two distinct niches (Wilson and Trumpp, 2006). An endosteal niche harbors quiescent HSCs and is defined anatomically by its immediate proximity to the endosteum (an inner bone surface) (Calvi et al., 2003; Kiel et al., 2005; Zhang et al., 2003). This niche is mainly composed of osteoblastic lining cells (expressing N-cadherin). By contrast, a vascular niche provides a site for HSC proliferation and differentiation (Kiel et al., 2005). This niche is located more centrally in BM cavities and mainly contains sinusoidal endothelial cells.

The mechanism by which BM niches support HSCs can be applied to protect leukemia-propagating cells (LPCs). This

Significance

Evasion of therapy by CPCs is one of the main obstacles in cancer treatment. The surviving CPCs are presumed to find "refuge" within preferential niches. We identified a protective niche within the BM that was created by CPCs in response to therapy in the mouse models of ALL. The niche was dynamically transient: beginning with Nestin$^+$ cells, maturing through their transition to α-SMA$^+$ cells, and ending with fiber residues. We found the niche was recurrent in BM biopsies of ALL patients who achieved only partial or nonremission after primary therapy, indicating that the niche formation may contribute significantly to the failure of achieving complete remission. Therefore, therapeutic strategies should seek to circumvent the formation of the early protective niche.

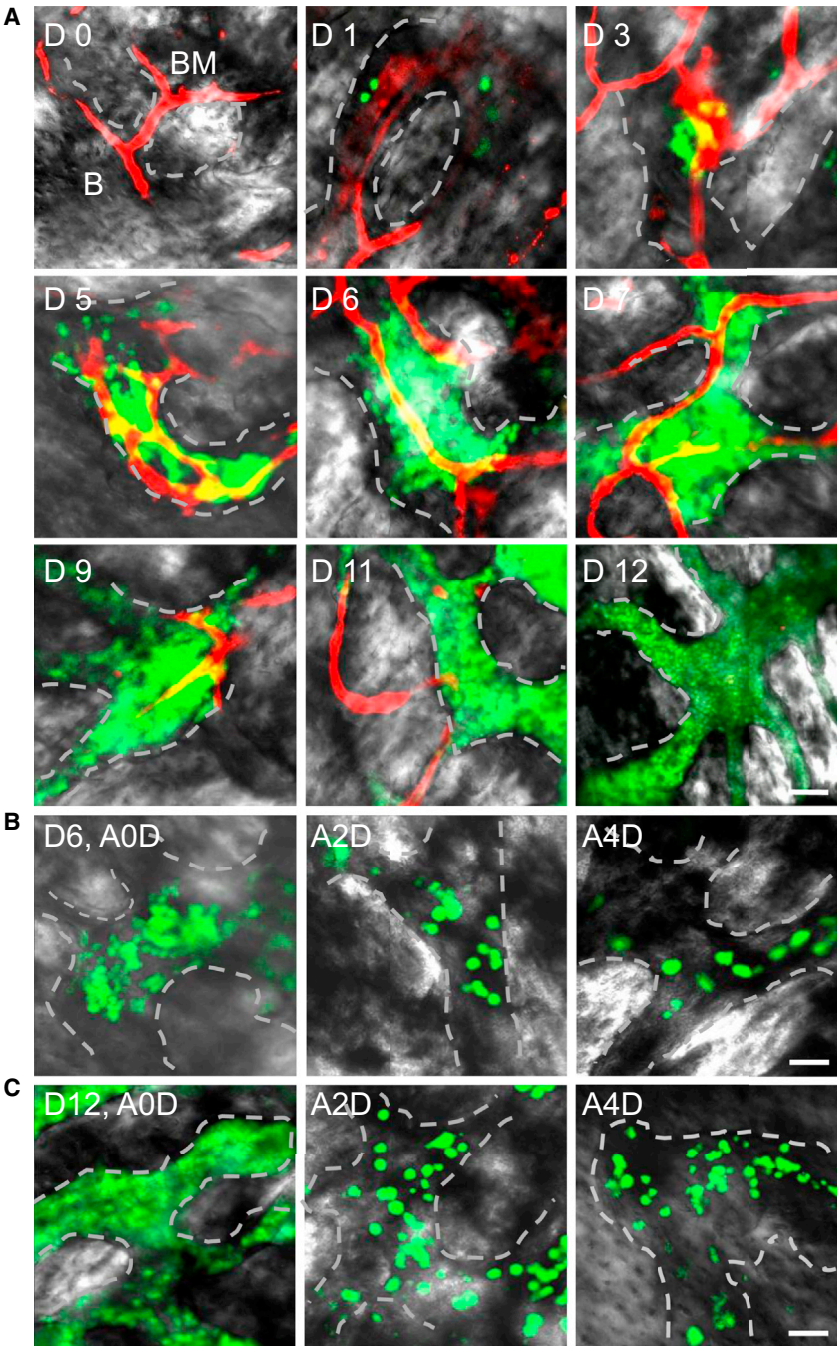

Figure 1. Ex Vivo Imaging of Leukemic Dissemination in Mouse BM

(A) Imaging of the residing and engrafting NALM-6-GFP cells in the BM. D0, D1, D3, D5, D6, D7, D9, D11, and D12 from day 0 to day 12 posttransplantation of leukemic cells. Red dye DiI indicates the vascular structure. Leukemic cells are green (GFP).

(B and C) Imaging of the residual cells in the mouse BM after chemotherapy. B, bone; BM, bone marrow; D6, D6 model; D12, D12 model; A0D, not treated with Ara-C; A2D, treated with Ara-C for 2 days; A4D, treated with Ara-C for 4 days. Scale bars represent 50 μm.

See also Figure S1 and Movie S1, S2, S3, S4, and S5.

mechanism has been investigated using a xenograft leukemia model. Leukemic cells reside and engraft in normal endosteal niches that are quiescent and resistant to chemotherapy (Ishikawa et al., 2007; Saito et al., 2010). However the scenario seems complicated, because other studies have observed that the growth of leukemic cells alters BM environments and interferes with normal hematopoiesis (Colmone et al., 2008; Schepers et al., 2013; Zhang et al., 2012). Therefore, the nature of nonmalignant supporting cells that form a BM niche for leukemic cells and the dynamic interplay between leukemic cells and niche components, particularly during primary therapy, remains unclear.

We addressed these issues by investigating in mouse transplantation models of acute lymphoblastic leukemia (ALL). We performed ex vivo imaging and immunoassays as well as investigated the patients' BM biopsies. The results reveal the interplay between LPCs and their nonmalignant supporting cells in the BM and the initial events resulting from primary chemotherapy.

RESULTS

Leukemic Dissemination Damages BM Microenvironments

We transplanted human leukemic cells into advanced immunodeficient animals to observe their behaviors in BM niches. Consistent with other studies (Notta et al., 2011), the engraftment and extent of leukemic dissemination were similar in NOD. CB17-Prkdc^{scid} /J (NOD/SCID) mice treated with anti-CD122 that depletes innate immune cells (NS122) or NOD/SCID mice harboring the deletion of the common gamma (γ) chain (NOG) (Anderson et al., 2011; Hong et al., 2008). Thus, the majority of our study was conducted using the NS122 model.

GFP-labeled NALM-6 cells, a human ALL cell line, was injected into sublethally irradiated mice via the tail vein. Afterward, we performed ex vivo imaging to observe the residence and dissemination of leukemic cells in BM niches (Figure S1A available online) (Xie et al., 2009). Vascular structures in the BM were visualized by red dye DiI. Images were captured on days 0, 1, 3, 5, 6, 7, 9, 11, and 12 after leukemic cells were injected. Sublethal irradiation damaged the vascular structures, but these structures were rapidly restored (days 0 to 3; Figure 1A) as previously reported (Hooper et al., 2009). Imaging on day 1 showed that leukemic cells resided in regions identified as endothelial microdomain juxtaposed to the