Ever-changing homes

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The concept of microenvironment recognizes the importance of cell extrinsic mechanisms in the regulation (or instruction) of cell behavior and is being increasingly explored in diverse fields, from cancer to neuroscience. Genetic manipulation together with new imaging methods, such as multiphoton microscopy, allowed the direct visualization of cell to cell interaction *in vivo* and, in part, the confirmation of early hypotheses about the existence of niches or microenvironments. The Portuguese immunologist Maria de Sousa described thymus-dependent (or T cell) areas in 1966¹ and made in 1971 the prescient observation that “*cell populations behave as if they have the ability to distinguish and home towards what is probably their usual environment*” (ecotaxis).² In 1978, Raymond Schofield proposed that cellular microenvironments in the bone marrow were associated with and critical to maintain tissue-fixed hematopoietic stem cells (HSCs),³ which have self-renewal and multipotency properties. Recent intravital microscopy studies have expanded on these hypotheses and showed that T cells migrate randomly within T-cell areas of the lymph node⁴ and that phenotypic HSCs are located in perivascular endosteal niches.⁵ The composition and role of different microenvironments is however incompletely understood. Although attractive, the hypothesis that niches are relevant for cancer development and chemoresistance is still poorly explored. We have recently applied high-resolution intravital microscopy of the bone marrow to address some of these questions. Combined two-photon and confocal microscopy of the murine calvarium was used to study the role of known microenvironments in aggressive blood cancers, namely acute T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML).

In the first study,⁶ we used a preclinical Notch1-driven T-ALL mouse model. We analyzed the whole-tissue three-dimensional distribution of single T-ALL cells at different disease stages, including early seeding, colonization/expansion, and relapse. We also time-lapsed these cells to study their behavior. Unexpectedly,

our unbiased approach revealed a stochastic distribution of T-ALL cells in relation to the three key bone marrow niches analyzed: osteoblasts, nestin⁺ mesenchymal stem/progenitor cells, and blood vessels.⁶ In contrast with the view that leukemic cells maintain long-lasting and stable interactions with their surroundings, we observed that T-ALL cells were highly migratory.⁶ Chemoresistant T-ALL cells, in particular, were extremely motile and exploratory within the marrow space.⁶ Altogether these observations revealed unique migratory features of T-ALL cells and argue against the existence of a single niche compartment in T-ALL.

In the second study,⁷ we asked the reverse question, that is, how the microenvironment is changed by leukemic cells. In AML, patients often present with severe cytopenias. We hypothesized that the remodeling of the bone marrow vascular microenvironment was contributing to the loss of nonmalignant hematopoiesis in AML. To explore the bone marrow ecology in detail, we performed intravital microscopy and high-resolution immunofluorescence of a mouse model of mixed lineage leukemia-AF9-driven AML. We observed that while central vessels were spared, bone-lining endosteal blood vessels were specifically eliminated during leukemia expansion.⁷ These endosteal transitional vessels are functionally unique and regulate both osteogenesis and hematopoiesis.⁸ Consistently, osteoblasts and HSCs were also lost in endosteal areas, during niche disruption by AML cells.⁷ When endosteal vessels were protected, we were able to partially recover HSCs and to delay disease relapse and increase survival.⁷ The latter is probably due to increased chemotherapy drug delivery to endosteal areas. Interestingly, we also observed that osteoblasts,⁶ but not endosteal vessels,⁷ are lost in T-ALL. This lineage-specific remodeling in leukemia may explain why blood cytopenias are more severe in AML. We propose that AML behaves as an invasive species, taking up space and destroying niches, thereby limiting the access of nonmalignant HSCs and hematopoietic competitors to *goods/nutrients* (eg, growth factors, chemokines). The driving factors and characteristics of T-ALL cell motility within the marrow space and the mechanisms underlying the vascular niche remodeling occurring in AML remain unresolved. Their study will contribute to our understanding of the *ever-changing* bone marrow microenvironments in leukemia.

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