Title: T-ALL: several homes rather than homeless?

Strapline: Dynamic T-ALL-niche cell interactions

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Microenvironment-targeted therapies are emerging as possible complementary approaches to treat malignancies. However, progress is hindered by the complexity of the interactions between malignant cells and their microenvironment. In leukemia, this complexity is further emphasized by a highly dynamic behavior of leukemic cells recently reported in *Nature*. Hawkins, Duarte and colleagues analyze leukemic cell localization relative to microenvironmental cells in T-cell acute lymphoblastic leukemia (T-ALL) through real-time intravital imaging[1]. Contrasting previous studies, Lo Celso and colleagues report a highly motile behavior and lack of stable interactions with specific microenvironmental cell types during disease progression or after chemotherapy. This study challenges some current concepts on microenvironmental dependence of leukemia.

Leukemic cells migrate throughout the body, then identify and seed suitable territories where they can expand. Cumulative evidence suggests that the support of the bone marrow (BM) microenvironment is required for leukemia initiation, progression or resistance to therapy. In some cases leukemic cells compete with their normal counterparts for the most permissive/supportive areas, since similar microenvironmental requirements have been proposed for normal hematopoietic stem cells (HSCs) and leukemia initiating cells (LIC)[2].

In a previous study, Lo Celso and colleagues developed powerful intravital microscopy methods which revealed that normal HSCs transplanted into irradiated mice (to stimulate BM homing) steadily locate near bone-forming cells

(osteoblasts)[3]. Osteoblasts also promote T cell development by providing the Notch ligand DLL4[4]. In non-irradiated mice, HSCs have been preferentially found near their niche cells, such as endothelial cells[5] and their associated BM mesenchymal stem cells (BMSCs)[6]. Hawkins, Duarte and colleagues take these notions further and use their optimized intravital microscopy to study leukemia and particularly the spatial relationships of T-acute lymphoblastic leukemia (T-ALL) cells with osteoblasts, nestin-GFP+ cells (marking BMSCs)[6] and endothelial cells.

Briefly, the position of transplanted T-ALL cells relative to osteoblasts, nestin-GFP+ cells and endothelium was recorded during 3-hour imaging sessions. Different time points after transplantation reflected various stages of leukemia development. A time-lapse study was performed after treatment with conventional chemotherapy to study the localization, division and migratory behavior of chemo-resistant T-ALL cells. This is an impressive effort that has provided the first extensive analysis of real-time leukemic cell positioning and migration within BM niches. A major novel finding is that T-ALL cells (either before or after chemotherapy) seem to be highly motile and lack any apparent localization preference within BM (Figure 1). The authors envision that targeting the interactions with microenvironmental cells (rather than specific cell types) is more likely to succeed therapeutically.

One key interaction of T-ALL cells with their BM microenvironment is mediated by the chemokine Cxcl12[7-9], highly expressed by nestin+ BMSCs, followed by osteoblasts and endothelial cells[6]. Two previous studies[7, 8] demonstrated that T-ALL cells require microenvironmental Cxcl12 for their BM lodgment and also for disease progression. Accordingly, increased cell surface expression of the Cxcl12 receptor Cxcr4 was found in T-ALL cells. It is possible that the dynamic behavior and heterogeneous Cxcr4 expression in T-ALL found by Lo Celso et al, together with different and variable Cxcl12 levels in the studied niche cells (nestin+ BMSCs>osteoblast & endothelial cells), might have masked some cell-type-specific interactions at the whole population level.

Whereas the study by Lo Celso's group does not show significant proximity of T-ALL cells to these Cxcl12-producing cells, the studies by Passaro, Pitt and

colleagues[7, 8] indicated cell-type-specific functional dependency of T-ALL. It is worth pointing out that proximity-based studies have revealed important regulators of HSC and progenitor function[3, 5, 6, 10] but have not always correlated with function in the aforementioned T-ALL studies. One example of discrepancy between proximity and function is found in the study by Passaro et al, where almost all T-ALL cells localized close to Lepr-traced mesenchymal cells (largely overlapping with nestin+ BMSCs) but Cxcl12 deletion in these cells did not affect T-ALL development. In contrast, endothelial Cxcl12 deletion rendered animals virtually disease-free, while only 70% of T-ALL cells appeared to locate near endothelial cells[8].

Observations made at different time points during disease development suggest that time scale might underlie discrepant conclusions and also preclude accurate comparisons across these studies. Whereas Passaro et al and Pitt et al focused on long-term effects and demonstrated that microenvironmental Cxcl12 is essential for T-ALL progression, the study by Lo Celso's group is mainly focused on imaging the early disease, starting from day 10 after transplanting T-ALL cells. It is possible that a posterior selection process might favor the expansion of Cxcl12-dependent clones sustaining the disease in the long term. In this regard, transformation of hematopoietic progenitors to T-ALL cells progresses through a phase of expansion of pre-leukemic clones driven by Notch signaling, and it has been suggested that disease progression requires further selection[11].

Importantly, although the three studies analyzed Notch-mutated T-ALL models, the mutations were different, leading to various kinetics and morbidity. The disease was comparatively less aggressive in the model used by Lo Celso's group[12]. Therefore, the microenvironmental dependence might also be less obvious than in the other studies, in which aggressive hijacking of the normal environment appeared to be required for leukemogenesis.

Another pioneering contribution from Hawkins, Duarte and colleagues is the visualization of T-ALL cells in the same mice before and after administration of different chemotherapy regimens. Unexpectedly, T-ALL cells increased their migratory activity upon administration of dexamethasone or vincristine and did not preferentially locate near osteoblasts or nestin-GFP+ cells. The lack of

sustained physical interactions with these niche cells made the authors hypothesize that T-ALL cells surviving chemotherapy do not require protective effects from the BM stroma. Remarkably, quiescence was not a hallmark of surviving T-ALL cells, since intense proliferation was observed immediately after chemotherapy.

On a broader scale, migration-related gene transcripts (including Cxcr4) were not differentially expressed by T-ALL cells before and after chemotherapy, raising the possibility that the dynamic interactions with the microenvironment might be guided by fast-acting changes in cell surface receptors, affecting for instance Cxcr4 recycling[7] or activation status. Increased Cxcr4 expression and migratory behavior was also observed by the Lo Celso group in HSCs after infection[13], suggesting potential commonalities that might facilitate the identification of new underlying mechanisms.

Altogether, the results from these studies argue for both key cell-autonomous mechanisms and promiscous/varied interactions with niche cells driving T-ALL invasion in BM which, as a Cxcl12-enriched environment, remains a favorite territory for T-ALL development. Further analysis of T-ALL cell trajectories during disease evolution might help to understand whether T-ALL cells stochastically migrate and interact with microenvironmental cells or whether specific patterns and selection occur driven by yet undefined stimuli.

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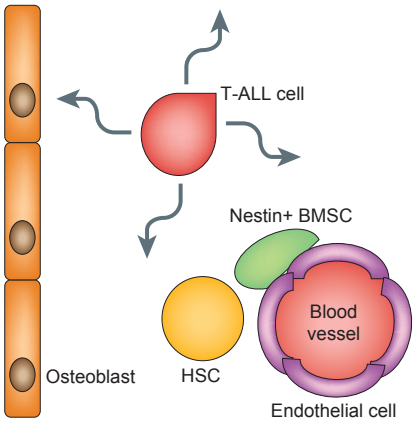
Figure 1. A simple representation of highly dynamic T-ALL behavior. Polarized cell shape illustrates high T-ALL motility while arrows pointing in different directions highlight lack of specificity in T-ALL positioning towards the presented HSC niche cells. T-ALL, T-cell acute lymphoblastic leukemia; HSC, hematopoietic stem cell; BMSC, bone marrow mesenchymal stem cell.


T-ALL cell
Nestin+ BMSC
Blood vessel
HSC
Osteoblast
Endothelial cell