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Biochemical heterogeneity and developmental varieties in T-cell leukemia

Arup K. Chakraborty[1,2] and Jeroen P. Roose[3,*]

[1]Departments of Chemical Engineering, Chemistry, Physics and Biological Engineering; Massachusetts Institute of Technology; Cambridge, MA USA; [2]Institute of Medical Engineering & Science; Massachusetts Institute of Technology; Cambridge, MA USA; [3]Department of Anatomy; University of California, San Francisco; San Francisco, CA USA

Molecular targeting of signaling pathways is being explored for many cancer types.[1] Success stories include inhibition of Abl kinase with Gleevec for chronic myeloid leukemia, or targeting of mutated, active B-Raf kinase with Vemurafenib in melanoma. However, there are many examples where this approach has failed, and cancer cells demonstrated effective mechanisms to overcome inhibition of the target. Heterogeneity within cancer cells can cause avoidance of inhibited nodes and selection of subpopulations of inhibitor-resistant cancer cells. Our recent studies of Ras signals in T-cell leukemia revealed a perplexing level of heterogeneity.[2]

The genetic make-up of cancer cell populations is one source of heterogeneity. Cancer-landscaping studies have uncovered that the main characters in oncogenic signaling networks can differ substantially between cancer (sub)types or within one tumor. Significantly, these efforts have aided better stratification of tumor types from patients and improved choice of therapy, such as for diffuse large B cell lymphoma (DLBL).[3] T cell acute lymphoblastic leukemia or lymphoma (T-ALL) cells demonstrate complex accumulation of cooperating oncogenic lesions,[4] creating genetic heterogeneity. Unlike for DLBL, no successful stratification methods exist to date for T-ALL.

Our recent study[2] focused on unraveling mechanisms that lead to aberrant Ras signaling in T-cell leukemia, a known affected pathway in approximately half of all T-ALL patients.[5] Using synergistic computational, genetic and biochemical methods, we uncovered at least two distinct and mutually exclusive genetic ways to trigger this oncogenic pathway. One type occurs through characteristic mutations in *RAS* genes found in many cancers that cripple the self-inactivating, GTPase activity of Ras. Second, we find that increased expression of the Ras activator *RasGRP1* (Ras guanine nucleotide exchange factor 1) is a frequent event in both pediatric T-ALL patients and in mouse models. Oncogenic *K-RAS*^{G12D} and dysregulated *RasGRP1* result in very different biochemically behavior. *K-RAS*^{G12D} T-ALL cells need to uncouple strong constitutive Ras signals from a P53-P21 cell cycle arrest program to grow. By contrast, *RasGRP1* T-ALL cells do not trigger cell cycle arrest, but efficiently activate Ras via receptor signal input from cytokines such as IL7 that are typically produced by bone marrow stromal cells (**Fig. 1**).

Development of more effective and less toxic therapies for T-ALL based on the underlying molecular pathogenesis is a high priority. Now that we have further identified Ras as a critical node in T-ALL, we face the same challenge as with many other Ras-driven cancer types. No effective inhibitors exist to block oncogenic *K-* or *N-RAS* or inhibit RasGRP1 activity. Ras is a signaling hub that connects to various effector kinase pathways, like the RasGTP-RAF-MEK-ERK and the RasGTP-PI3K-AKT cascades.[1] Whereas kinases with their conserved ATP-binding pocket are ideal targets for molecular therapy, we observed a perplexing level of heterogeneity in the activity through these kinase effector pathways. Moreover, clonal T-ALL lines can switch pathways when signals through the RasGRP1 node are decreased, a form of biochemical plasticity. Thus, kinase pathways downstream of Ras are not "locked" but are heterogeneous and plastic, which complicates the development of a biochemical roadmap to design targeted therapies with kinase inhibitors for T-ALL.

Lastly, T-ALL display a variety in developmental stages. Our clonal *K-RAS*^{G12D} and *RasGRP1* T-ALL lines also demonstrate distinct developmental cell surface marker patterns determined by FACS. It is not clear whether T-ALL cells mirror distinct stages of the developmental program of normal T lymphocytes and if T-ALL follow a forward progression. A novel single-cell mass cytometry (CyTOF) method that allows simultaneous analysis of more than 30 markers suggests that the developmental issue may be more complicated than previously anticipated. CyTOF analysis of bone marrow cells has revealed that normal hematopoiesis is a continuum with over a hundred identifiable subsets.[6] It is very likely that blood cancers will also display continua, further increasing the variety.

Our leukemia studies summarized above greatly benefited from our computational efforts to formulate meaningful hypothesis on the leukemic Ras signaling pathways.[2] Similarly, computational models proved useful partners for our experimental investigation of the biochemical mechanisms that underlie responses of normal T cells to stimuli.[7,8] Signaling in T cells is highly cooperative with nonlinear biochemical events. The cooperative nature of signaling processes makes it difficult to intuit underlying mechanisms from experimental observations. In addition, many biochemical events are inherently stochastic in character. Stochastic computational models of signaling events yield the consequences of specified hypotheses and can thus be used to eliminate hypotheses that appear plausible, but are incorrect because intuitive

*Correspondence to: Jeroen Roose; Email: jeroen.roose@ucsf.edu
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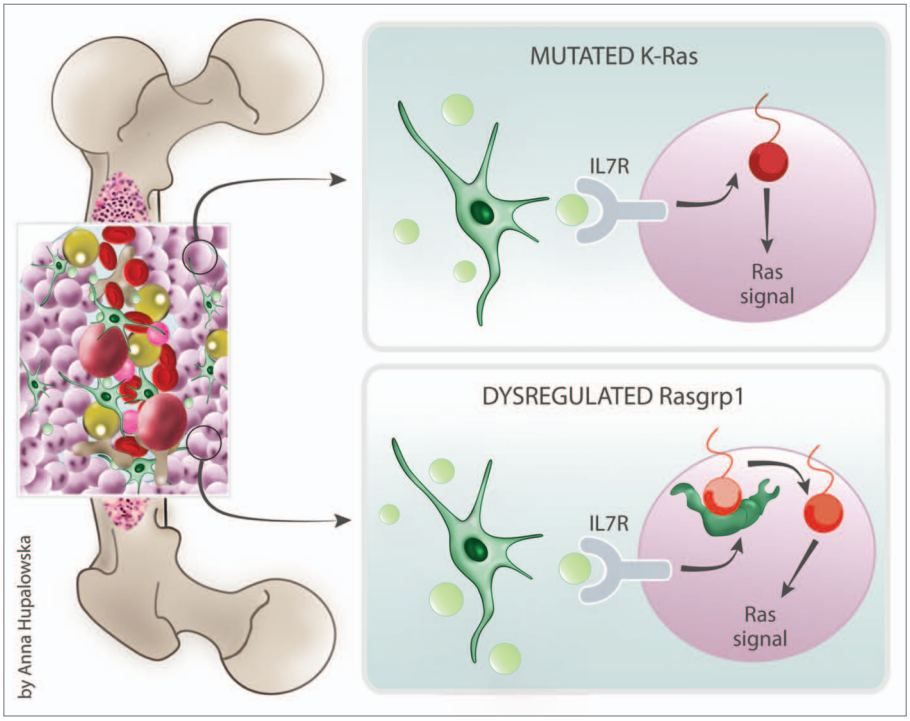

Figure 1. T-cell leukemia cells expand in the bone marrow in response to growth factors like interleukin 7 (IL7 in green) and take over the space in the cavity (uniform purple cells in the illustration). This expansion leads to a loss of the variety in bone marrow cells, such as blood stem cells (in pink), red blood cells (in red) and fat cells (in yellow) that are normally seen in the bone marrow. The Hartzell et al. study describes how two related, but distinct, genetic alterations in T-cell leukemia cells, mutated K-Ras or dysregulated Rasgrp1, both lead to T-cell leukemia by responding to IL7 and other signals in different manners. Graphics by Anna Hupalowska.

consideration of the cooperative processes can be flawed. Moreover, these models can identify missing knowledge and explore the consequences of diverse hypotheses and design experiments to test these. It is critical that these computational efforts are paired with biochemical investigations in which the cell biological signals can be resolved at the individual cell level, so that nuances in biochemical signals between subpopulations of cells within a pool can be appreciated.[8] We envision that combining computational, developmental and biochemical approaches will be useful to better understand oncogenic signaling pathways at single cell resolution, so that it can resolve the features of heterogeneity and plasticity.

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