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HEMATOPOIESIS AND STEM CELLS

Comment on Kull et al, page 99

Incorporating signaling dynamics into fate decision

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In this issue of *Blood*, Kull et al[1] demonstrate that nuclear factor κB (NF-κB) signaling dynamics are variable among purified hematopoietic progenitors, such as the granulocyte-macrophage progenitors (GMPs). In response to the same inflammatory signals, GMPs destined to become macrophages (GMP^{M}) tend to display multiple rounds of NF-κB nuclear-cytoplasmic translocations (oscillating, OSC), whereas their immediate bipotent predecessors (GMP^{GM}) mostly undergo 1 single round of NF-κB activation (transient, TRA). Forcing OSC on the bipotent GMP^{GM} cells directs their fate trajectory more toward becoming macrophages.

Situated downstream of the hematopoietic stem cells and multipotent progenitors, GMPs are highly proliferative and poised to becoming granulocytes or monocytes/macrophages, major components of the innate immune system. Their proliferative prowess represents a critical relay point from the few, mostly quiescent hematopoietic stem cells to the much more numerous myelomonocytic descendants that are constantly turning over, especially during injury repair.[2] Churning out large numbers of cells quickly is only half of their mission: these newly produced cells also need to adopt the correct identity. How is cell identity determination tailored to the type, phase, and magnitude of homeostatic pressure posed by the myriad forms of injury or infection? Previous work on hematopoietic progenitor fate specification focused on transcription factors (TFs), such as PU.1-GATA1[3] or Gfi1-IRF8.[4] Binary fate choices at bifurcation points can be explained by a mutual antagonism between a pair of TFs. What is missing in the dueling TF model is what determines the outcome; if the duel is left to its own, the outcome would be rigid, like a coin toss (equally divided all the times). The work by Kull et al places NF-κB dynamics onto this dueling scene, so that the 2 types of progeny could be produced under this dynamic in all possible proportions.

The report by Kull et al offers 3 important messages. First, it is the nature and pervasiveness of cellular heterogeneity. With the widespread use of single-cell analytic tools, heterogeneity is omnipresent. But what does heterogeneity mean when cells are known to be similar to each other? Obvious explanations for cellular heterogeneity could include the environment or extrinsic signals, and/or genetic, epigenetic, or oscillators (eg, cell cycle, circadian clock).[5,6] Strikingly, Kull et al reveal that even among purified cells of a narrow differentiation stage (ie, the GMPs), their NF-κB dynamics differ. Further, when all the obvious variables are controlled for (ie, by treating purified GMPs with the same signaling inducer, tumor necrosis factor-α) response from these otherwise similar cells remained variable, as shown by their different NF-κB dynamics. Therefore, an important aspect of heterogeneity is the cellular response as a function of time. In other words, the same cell could appear different when measurements are performed at discrete time points. Of note, the cell cycle was ruled out as a significant contributor to the observed heterogeneity, a finding contrary to those reported by single-cell datasets.[4] Second, can the difference in NF-κB dynamics be captured and described by concrete differences in gene expression? To address this question, the authors used Trackseq, a powerful new approach that allows transcriptomic analysis after imaging. Equipped with the ability to interrogate the transcriptome of individual TRA and OSC cells, the authors teased out a set of genes whose expression differed between the TRA and OSC cells, with most being higher in OSC cells. Third, does the observed heterogeneity have biological consequences? Kull et al used microfluidics to force-feed inflammatory signals at precise time points. Indeed, the GMPs responded differently to the signals. Forced oscillation in GMP^{GM} biased cell fate toward GMP^{M} even though the effect size appears small under the experimental condition. Different inducer (tumor necrosis factor-α and interleukin-1β) yields different proportion of OSC and TRA cells; interleukin-1β stimulation leads to more TRA cells.

Kull et al's work also suggests how the physical parameter, time, is interpreted and communicated on 2 distinct biological scales, between individual cells and the entire organism. Although there is much interest in turning back time for the organism,[7] what does time mean for an individual cell, which is a miniature biochemical reactor adhering only to the laws of chemistry and physics not caring for our notion of "slow down" or "stay young"? Organismal aging is associated with myeloid-biased hematopoiesis, with inflammation playing an important role.[8] Could the myeloid-biased hematopoietic stem and progenitors popping up in our old age be displaying peculiar NF-κB dynamics? Can forcing specific NF-κB dynamics change the fate outcome of hematopoietic progenitors in vivo? Can specific NF-κB dynamics be achieved

therapeutically? Because the dynamics was only observed in artificial culture conditions, even though similar dynamics likely occur in vivo, investigating such dynamics in vivo awaits more sophisticated tools. On a fundamental level, a pressing question that is not addressed in the report by Kull et al is what determines NF-κB dynamics in response to the same signals. The authors described observations, such as larger nuclear area or shorter cell cycle, in association with specific NF-κB dynamics; is this only an association or is there a specific mechanism linking them? Last, NF-κB is only one of several common oscillating dynamics responding rapidly to environmental signals. In this regard, the role of ERK dynamics in cell fate determination has been widely reported, including in hematopoietic stem and progenitors.[9,10] Because oscillating behavior inherently implies or measures time, integrating oscillating dynamics could be a general theme for how individual cells interprets time, so that what they are now and what they become next could be coordinated in sync with the need of the tissue or the organism.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2022016420

LYMPHOID NEOPLASIA

Comment on Shanafelt et al, page 112

Ibrutinib frontline in young patients with CLL

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In this issue of *Blood*, Shanafelt et al[1] confirm the continued superiority of ibrutinib plus rituximab (IR), compared with the prior standard treatment, fludarabine, cyclophosphamide, and rituximab (FCR), for fit patients with chronic lymphocytic leukemia (CLL). In addition, they report relevant data on the tolerability of continuous treatment with BTK inhibitor.

More than 17 years ago, the FCR chemoimmunotherapy regimen was developed by the MD Anderson Center (Houston, TX)[2] and was later shown to be superior to chemotherapy alone.[3] Later, extended follow-up data showed that this regimen in younger, fit patients had the potential for long-lasting disease control, possibly even cure, in a subgroup of patients with favorable prognostic profile.[4,5] In contrast to FCR, chemoimmunotherapies based on less-intensive chemotherapy backbones, such as chlorambucil or bendamustine, did not show similar long-lasting remissions in more elderly and less fit patients. BTK inhibitors, alone or in combination with anti-CD20 antibodies, had been shown to be superior to those less intensive treatment regimens in elderly or unfit patients with CLL.[6,7]

In the E1912 study, the ECOG-ACRIN study group reported that, at a median of 34 months, the IR regimen was superior to FCR. Now, a follow-up of nearly 6 years clearly confirms the superiority of IR with respect to progression-free survival (PFS) (5-year PFS rates for IR, 78% and for FCR, 51%; HR [hazard ratio], 0.37; 95% confidence interval [CI], 0.27-0.61; $P < .0001$). Notably, even the subgroup of patients with mutated immunoglobulin heavy chain (IGHV) status, which benefitted most from the FCR regimen,[4,5] had an HR of 0.27 (95% CI, 0.1-0.62) for PFS. Although overall survival (OS) for the IR-treated group was still superior with longer follow-up, the difference was less than in the previous report. A subgroup analysis for OS showed that only patients with unmutated IGHV status benefited from IR, a finding limited by the reduced power of this secondary analysis (HR 0.35 for OS in patients without mutated IGHV (95% CI, 0.15-0.80) vs HR 0.72 in those with a mutation (95% CI, 0.15-3.47). The decreasing difference in OS may be related to greater use of targeted agents in relapsed disease than during the first study.[8] However, because data for relapse treatment were available only for patients dying of CLL or Richter transformation (see supplemental Table 3A in Shanafelt et al), this hypothesis cannot be confirmed by complete data analysis of all salvage therapies.

The outcome of patients who discontinued ibrutinib treatment because of adverse events was also presented. These data are highly relevant for clinical management. Seventy-seven patients (21.9% of all patients from the IR arm) discontinued BTK inhibitor therapy after a median time of 25.9 months, because of adverse events or complications. The update shows that the median time from ibrutinib discontinuation to disease progression was 25 months. Although the difference was not statistically significant, the tendency was for longer duration of ibrutinib therapy, particularly treatment exceeding 1 year, to result in longer disease-free survival after treatment discontinuation. Moreover, despite the difference in treatment duration, the IR