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Microsized inflammaging protects stem cells

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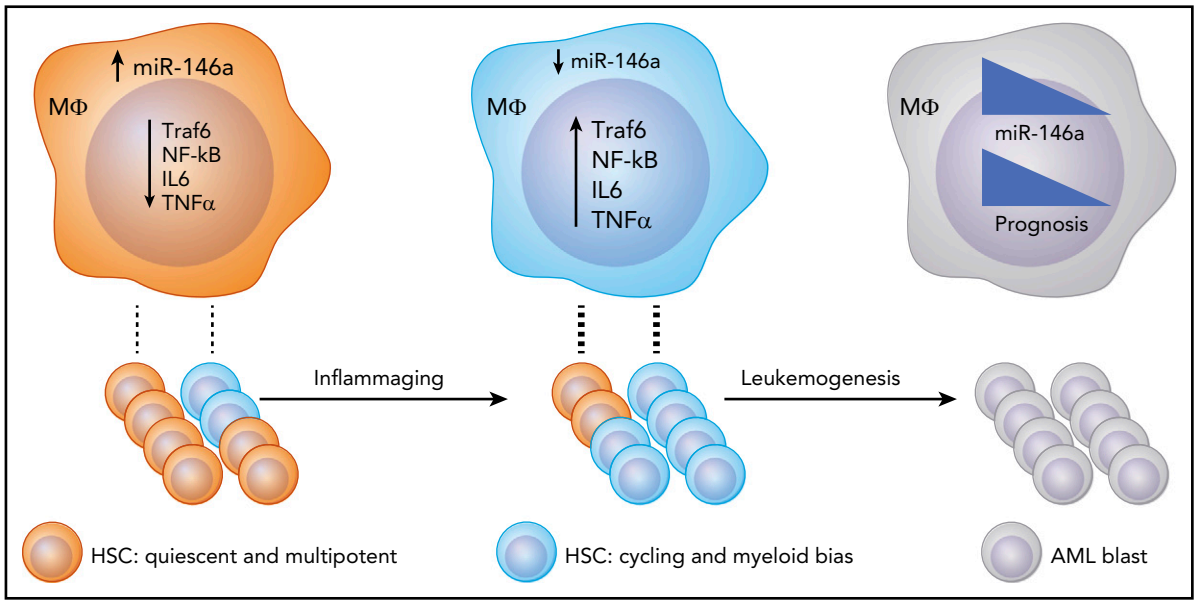
In this issue of *Blood*, Grants et al have identified connections between reduced expression of microRNA-146a (miR-146a), increased inflammation, impaired hematopoietic stem cell (HSC) quiescence, and a poor prognosis for acute myeloid leukemia (AML).[1] miR-146a was originally identified as an anti-inflammatory microRNA that targets signaling proteins, which mediate inflammatory responses.[2,3] Deletion of miR-146a in mice phenocopies many aspects of age-dependent inflammation,[2,4] termed "inflammaging."

Inflammaging impacts a plethora of human disease conditions, including obesity, diabetes, arthritis, heart disease, and cancer.[5] However, the molecular mechanisms underlying this chronic pathological state, and how they contribute to blood cancers, remain poorly understood. In this study, the authors identify low miR-146a as a poor prognostic marker in AML and find its expression to be decreased in older patients, as well as in aging mice (see figure). The authors then use *MiR-146a*$^{-/-}$ mice, which develop myeloid malignancies in some cases,[2,6] to carefully assess the impact of inflammaging on HSC phenotypes utilizing a variety of functional and state-of-the-art genomics approaches to monitor single cells.

Previous work has demonstrated that HSCs from miR-146a–deficient mice are dysfunctional.[2,6] The current study clarifies that this is due to alterations in epigenetic modifications that activate inflammaging gene expression programs. These epigenetic and gene expression patterns reflect the underlying heterogeneity of the HSC pool. The composition of the HSC pool changes in miR-146a–deficient mice. The authors' careful single-cell experiments show an initial "programmed" difference in cell division in miR-146a–deficient HSCs, which depletes primitive HSCs and causes an increase in myeloid progenitors, including possible leukemia stem cells.

The authors also make a convincing case that miR-146a expression is important in AML prognosis. miR-146a expression is not correlated with cytogenetic abnormalities or mutations that confer a prognostic impact. Rather, the effect of miR-146a is due to its role in regulating chronic inflammation. During aging, inflammatory changes accumulate, and there is a loss of HSC stemness and bias toward myeloid cell development.[7] miR-146a appears to maintain "stemness" in the most primitive HSC compartment, and its loss in mice phenocopies the inflammaging phenotype, including a myeloid skewing. The mechanism of stemness maintenance involves suppression of inflammatory cytokines, including interleukin-6 and tumor necrosis factor-α, which are primarily produced by mature hematopoietic cells, such as macrophages, and shown to be functionally linked to miR-146a hematopoietic phenotypes. Interestingly, wild-type HSCs acquired some features of miR-146a$^{-/-}$ HSCs when they were cotransplanted, indicating a significant effect by factors extrinsic to the wild-type HSC and consistent



Proposed model: as a consequence of inflammaging, miR-146a levels are reduced, and inflammatory cytokine production is increased in hematopoietic cells, including macrophages. Consequently, these cytokines promote HSC proliferation and differentiation into stem cells with a myeloid and, potentially, leukemic bias. This state contributes to malignant transformation by some cell clones. Upon transformation to AML, miR-146a expression negatively correlates with prognosis.

with a cytokine-mediated mechanism of action. Hence, it will be interesting to determine if miR-146a is a determinant of initial leukemic transformation in conjunction with driver mutations that have been well documented.[8]

This interesting work also raises additional questions. Is there a difference in hematopoietic miR-146a expression between AML patients and age-matched controls? Studies in autoimmune disease have demonstrated polymorphisms in the promoter of miR-146a, which reduce its expression.[9] It will be interesting to determine if this is the key to reduced miR146a expression during aging and in the setting of AML. Another open question is whether the difference in prognosis in AML is related to the direct sensitivity of AML blasts to inflammaging signaling or whether non-AML "inflammaged" HSCs are less able to recover within the bone marrow following therapy. Furthermore, the reduction in HSC function appears to be the result of both cell-intrinsic and cell-extrinsic mechanisms. The extent to which each mechanism contributes to HSC dysfunction is an exciting area for future investigation. Answers to these questions should guide future therapeutic strategies, including replenishing miR-146a, which initial evidence suggests is possible.[10]

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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LYMPHOID NEOPLASIA

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Inverting the BTK-BCL2 order

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In this issue of *Blood*, Lin et al report the first long-term follow-up data showing that Bruton tyrosine kinase inhibitors (BTKi's) are effective in chronic lymphocytic leukemia (CLL) after previous progression on venetoclax.[1]

Clinical development of the BTKi ibrutinib preceded that of the BCL2 inhibitor venetoclax, with approval for use in relapsed CLL in February 2014. The initial approval of venetoclax came in April 2016 for the limited group of previously treated CLL patients with 17p deletion. Given this timing, a prospective clinical trial was rapidly performed that established the effectiveness of venetoclax in patients with disease progression during or after ibrutinib.[2] Since then, approval has been extended for venetoclax, in combination with rituximab for any CLL patient who has had at least 1 previous therapy,[3] and in combination with obinutuzumab for previously untreated CLL patients.[4] These venetoclax regimens have certain advantages: they have a defined duration, are well-tolerated, and achieve deep remissions with undetectable minimal residual disease (MRD). The inevitable question then arises: will BTKi's work well after venetoclax, that is, can the order of therapy be inverted, using venetoclax as a first targeted agent? The hesitation comes from the unknown efficacy of BTKi's after treatment with venetoclax has failed.

The article by Lin et al seeks to fill this knowledge gap. They present the outcomes of BTKi therapy in 23 patients previously treated on 1 of 4 early venetoclax trials and whose CLL progressed during ongoing venetoclax therapy. The patients were all heavily pretreated, with a median of 4 previous regimens that included fludarabine-cyclophosphamide-rituximab in almost all cases, and no previous BCRi exposure. *TP53* abnormalities were present in 76% of patients and complex karyotype was present in 68% of patients. The patients had a median duration of response on previous venetoclax therapy of 29 months, mostly partial responses. Twenty-one patients went on to receive ibrutinib, and 2 patients went on to receive zanubrutinib, with an overall response rate (ORR) of 91%. Their median progression-free survival (PFS) was 34 months when being treated with a BTKi; 11 patients with a median follow-up of 33 months were still receiving therapy, and 12 discontinued therapy (8 for progression and 4 for toxicity) (see figure). Median overall survival was 42 months.

Although the Lin et al study is a retrospective single-institution study with a limited sample size, overall, these data are reassuring because the ibrutinib results seem largely comparable to those reported in the most similar patient population previously studied: the phase 1b/2 study of ibrutinib.[5] That study