idelalisib in cells from patients with both resistant and susceptible disease, consistent with the lack of compensatory mutations in the PI3K pathway. Moreover, treating cells with idelalisib and MEK inhibitors led to enhanced inhibition of cell survival and proliferation. These results therefore suggest that the addition of an MAPK pathway inhibitor can overcome adaptive resistance to idelalisib.

Patients with CLL now have several novel therapies available to them. In addition to idelalisib and ibrutinib, venetoclax, which blocks BCL2 function, has been approved.[9] Idelalisib has been plagued with adverse effects, including colitis and pneumonitis.[10] There may therefore be little appetite for combining idelalisib with another inhibitor that can add to the list of adverse effects. However, second-generation inhibitors potentially with higher selectivity and/or fewer adverse effects are also being developed, and several have now been approved.[11] Moreover, emerging evidence suggest that PI3Kδ inhibitors may be used intermittently and may reduce adverse effects while retaining therapeutic effects. Following this logic, sequential use of inhibitors may also be an attractive option. This approach would require new clinical trials, as MAPK inhibitors have not yet been approved for CLL.

Why are mutations activating this pathway not found in CLL when they are so common in other cancers? Why is drug resistance not associated with such mutations? Generating hypotheses based on the absence of evidence is a challenge, but these 2 studies are starting to suggest that, although normal PI3K signaling is essential for CLL, hyperactivated PI3K signaling may be selected against. Moreover, if CLL cells can compensate with PI3K inhibition by increasing MAPK signaling, does it mean that PI3K-δ–dependent MAPK activation is essential for CLL? The other possibility is that the PI3K and MAPK pathways share a common target. In this context, it is worth noting that both the PI3K and MAPK pathways can converge after activation of mTOR and its downstream substrate, S6K.

Both BTK and PI3Kδ inhibition act in part by purging CLL cells from their protective interactions with the stroma in the lymph nodes. This action leads to the characteristic lymphocytosis observed shortly after treatment and renders the CLL cells more susceptible to apoptosis, which can be accelerated by coadministered therapeutics, such as rituximab or chemotherapy.[2] At present, it is not known whether the MAPK pathway can also trigger lymphocytosis, but this possibility should be monitored closely in future trials.

The results in Murali et al highlight a key role for MAPK pathway activation as a resistance mechanism, suggesting that MAPK pathway inhibitors may be considered for patients who no longer respond to PI3K-δ inhibitors.

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LYMPHOID NEOPLASIA

Comment on Dumontet et al, page 57

Extracellular vesicles prime the bone marrow niche

Franziska Haderk | University of California, San Francisco

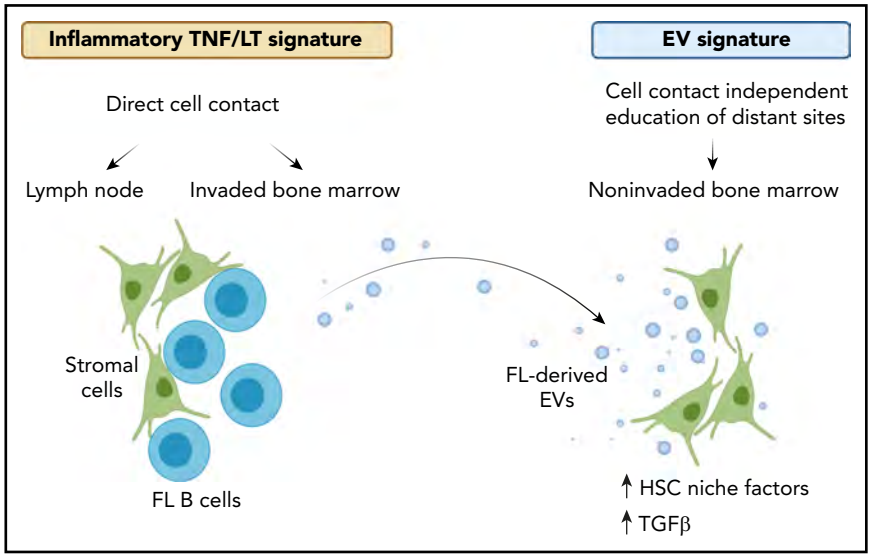
In this issue of *Blood*, Dumontet et al[1] highlight the role of cancer cell-derived extracellular vesicles in forming a tumor supportive stromal cell niche in follicular lymphoma.

Extracellular vesicles are small membrane-encapsulated vesicles that harbor important information such as RNA and proteins derived from their cell of origin. Their role in priming the premetastatic niche has been well recognized in solid cancers. This is exemplified by their ability to induce endothelial permeability and recruit bone marrow progenitor cells and by driving metastasis formation in an organotrophic manner.[2,3] In hematologic malignancies, cancer cell–derived extracellular vesicles are equally important in the generation of a tumor-supportive microenvironment. Among others, they have been reported to mediate the transition of stromal cells to cancer-associated fibroblasts in chronic lymphocytic leukemia.[4]

Follicular lymphoma is an indolent lymphoma with lymph node and bone marrow involvement, the latter presenting an

adverse risk factor and being crucially dependent on active homing of malignant cell subclones to the bone marrow niche.[5,6] However, cell–cell communication networks, which are involved in the selection and homing of certain malignant B cell subclones to the bone marrow, remain to be explored in more detail. Similarly, the role of extracellular vesicles in forming the bone marrow niche in follicular lymphoma is an important area of current investigation.

In the presented work, Dumontet et al introduce cancer cell–derived extracellular vesicles as an important player within the follicular lymphoma bone marrow microenvironment (see figure). The authors isolate extracellular vesicles from conditioned media of follicular lymphoma cell lines, primary follicular lymphoma B cells, and patient-derived blood plasma. Using healthy donor–derived mesenchymal stromal cells, they identify a rapid uptake of extracellular vesicles in stromal cells and a vesicle-mediated increase in stromal cell–mediated survival support for follicular lymphoma cells. Given prior reports on lymphoid-like stromal cell differentiation induced by stimulation with tumor necrosis factor-α and lymphotoxin-α1β2 (TNF/LT), the authors put vesicle-mediated effects (EV signature) in context with TNF/LT-triggered alterations in stromal cells. They successfully dissect differential gene expression profiles of the 2 lymphoma-supportive stromal cell stimuli. Of note, there is limited overlap between EV- and TNF/LT-induced gene expression signatures in stromal cells. Comparing observed gene expression changes to relevant tissue sites for disease manifestation, the TNF/LT signature shows enrichment in mesenchymal stromal cells of lymphoma cell–invaded lymph node and bone marrow sites, whereas the EV signature shows enrichment in mesenchymal stromal cells of patient-derived noninvaded bone marrow. Similarly, coculture assays of malignant B cells with mesenchymal stromal cells show an overlap with expression changes observed in invaded bone marrow. This indicates that the TNF/LT signature resembles pathologically relevant alterations of the tumor microenvironment where stromal cells are in close contact with follicular lymphoma cells. On the other hand, vesicle-mediated effects overlap with stromal cell subsets in the bone marrow that resemble components of the hematopoietic stem cell niche. Thus, it is likely that priming of the bone marrow site via extracellular vesicles allows for later infiltration of follicular lymphoma cells. The authors provide further evidence pointing to a vesicle-induced expression of genes centrally important for the formation and maintenance of the hematopoietic stem cell niche and osteolineage and adipogenic differentiation. Focusing on signaling alterations triggered by extracellular vesicles, a dominant role for transforming growth factor β (TGFβ) becomes apparent. As such, higher levels of TGFβ1 and TGFβ3 in vesicle-primed mesenchymal stromal cells are reported. A detailed characterization of transcription factors encapsulated by extracellular vesicles or driving the EV signature in stromal cells points to a central role of the TGFβ signaling pathway. Follicular lymphoma–derived extracellular vesicles trigger canonical TGFβ-SMAD and noncanonical TGFβ-p38 signaling: both are proven reversible by the TGFBR1 inhibitor galunisertinib. In addition, TGFβ-independent enrichment for signal transducer and activator of transcription 6 is observed in follicular lymphoma–derived extracellular vesicles.



Gene expression alterations in stromal cells vary across tissue sites of disease manifestation in follicular lymphoma. At sites of direct cell contact, namely lymph node and invaded bone marrow, an inflammatory TNF/LT-mediated expression signature in stromal cells provides survival support for malignant B cells. In addition, follicular lymphoma cells release extracellular vesicles that can prime stromal cells at distant sites in a cell contact–independent manner. Thus, gene expression in noninvaded bone marrow stromal cells derived from follicular lymphoma patients correlate with EV-mediated alterations. The latter is characterized by an upregulation of HSC niche factors and TGFβ signaling in stromal cells. FL, follicular lymphoma; HSC, hematopoietic stem cell. Created with BioRender.com.

Altogether, the authors generate new insights regarding the different expression states of bone marrow stromal cells across sites of disease manifestation. They successfully dissect driving forces for the respective alterations, focusing on cell contact, TNF/LT-mediated vs cell contact–independent, extracellular vesicle–mediated stimulation. The relevance of context-dependent remodeling of the tumor microenvironment is addressed by highlighting differential cell–cell communication networks that are established between follicular lymphoma cells and bone marrow stromal cells primed with TNF/LT or vesicle stimuli, respectively. Differences between lymph node and bone marrow–located follicular lymphoma cells indicate the bone marrow niche as a site that harbors malignant B cells that are less proliferatively active but show the expression of drug resistance–associated genes.

Understanding tumor microenvironment alterations that allow the dissemination of malignant cells throughout the body and foster their survival is of utmost importance to design rational treatment strategies. Recent success of ibrutinib as a pathway-targeted inhibitor in hematologic malignancies has emphasized the benefit of inhibiting malignant B cells and simultaneously limiting their access to a tumor supportive microenvironment.[7] As such, ibrutinib treatment results in a release of malignant cells from secondary tissue sites into the peripheral blood in chronic lymphocytic leukemia: a phenomena that

potentiates treatment success.[7] Extracellular vesicle–mediated TGFβ signaling in bone marrow mesenchymal stromal cells, as presented by the authors, may represent another important, targetable axis in the establishment of a cancer cell favorable tissue niche and may impact treatment response in follicular lymphoma.

Similarly, TGFβ-positive stromal cells have recently been associated with resistance to cancer immunotherapy.[8] A role of extracellular vesicles in forming a tissue niche that provides protection of cancer cells from targeted therapy and immunotherapy may be important to explore in the future, and new insight may guide the design of combinatorial treatment strategies.

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MYELOID NEOPLASIA

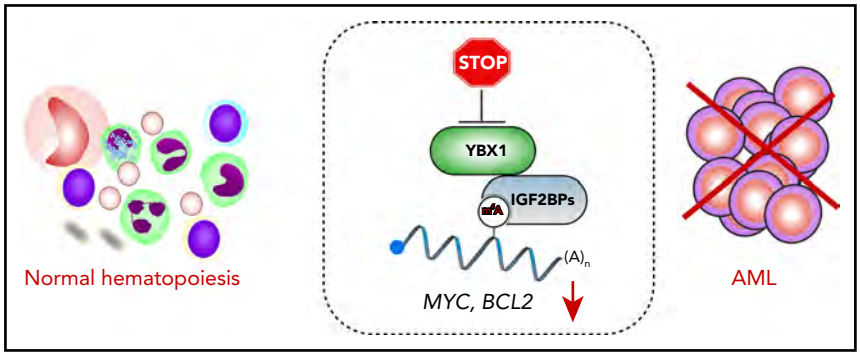
Comment on Feng et al, page 71

The essential reading list for AML: the m^{6}A transcripts

Kamil R. Kranc | Queen Mary University of London

In this issue of *Blood*, Feng et al reveal that the RNA-binding protein YBX1 is essential for acute myeloid leukemia (AML) development and propagation but is not critical for normal hematopoietic stem cell (HSC) functions and multilineage hematopoiesis.[1] Mechanistically, the authors propose that YBX1 promotes AML by interacting with IGF2BPs, readers of messenger RNAs (mRNAs) that are chemically modified by methylation at the N6 position of adenosines (m^{6}A), to stabilize these transcripts. Among these stabilized transcripts are those encoding MYC and BCL2, key regulators of AML cell proliferation and survival.

Given that long-standing therapeutic strategies in AML often fail to fully eliminate leukemic stem cells that fuel disease and are ultimately responsible for fatal relapses, the AML community has turned to new fields in search of novel efficient therapeutic targets. Although the role of gene transcription in leukemogenesis has been studied extensively, the functional significance of posttranscriptional regulation of gene expression, including RNA modifications, is only beginning to emerge. Recent evidence indicates that m^{6}A, the most abundant internal mRNA modification, is an important regulator of normal and malignant hematopoiesis.[2-7] The m^{6}A modification is installed in the proximity of stop codons and 3′ untranslated regions by the m^{6}A methyltransferase complex ("m^{6}A writer") and can be removed by m^{6}A demethylases (collectively called "m^{6}A erasers").[8] The functions of the m^{6}A modification are predominantly executed by YTH domain-containing m^{6}A readers (including nuclear YTHDC1, and cytoplasmic readers YTHDF1-3 and YTHDC2). Although nuclear YTHDC1 regulates mRNA splicing and nuclear export, cytoplasmic m^{6}A readers collectively promote decay and translation of m^{6}A-modified mRNAs. Furthermore, a recently discovered class of cytosolic readers, insulin-like growth factor 2 mRNA-binding proteins 1-3 (IGF2BP1-3), functions to stabilize m^{6}A-modified mRNAs.[9] Significantly, m^{6}A writers and erasers are overexpressed in AML, and their inactivation cripples AML cells through multiple m^{6}A-dependent mechanisms.[4-7] Furthermore, the mRNA m^{6}A reader YTHDF2 is highly expressed across human AML, and its inactivation selectively compromises AML development and propagation by extending the half-life of m^{6}A-modified transcripts, including TNFR2, thus sensitizing them to apoptosis.[3]



YBX1 interacts with the m^{6}A reader IGF2BP proteins to stabilize m^{6}A-modified transcripts, including *MYC* and *BCL2*. Upon YBX1 inactivation, *MYC* and *BCL2* undergo accelerated decay, thus compromising AML cells. However, the loss of YBX1 has no major impact on normal multilineage hematopoiesis.