signaling in these lymphomas by the SYK inhibitor R406 induces apoptosis, which is primarily mediated by induction of the proapoptotic BCL2 family member, HRK.5

In this issue of Blood, Szydłowski and colleagues further characterize the components of the proapoptotic pathway that is repressed by the tonic BCR signal.4 They show that inhibition of the tonic BCR signal results in increased activation of FOXO1 and increased expression of its target genes, including the proapoptotic BCL2 family member, BCL2L11, and the cell-cycle inhibitor p27. Importantly, they also show that depletion of FOXO1 by RNA interference abrogates R406-induced cytotoxicity in DLBCL cells with tonic BCR signaling, but not in cells with chronic-active BCR signaling. The reduced sensitivity to R406-induced apoptosis in cells with depleted FOXO1 is associated with reduced induction of HRK, further suggesting that FOXO1 is the critical mediator of the proapoptotic pathway that is repressed by the tonic BCR signal. Finally, Szydłowski et al investigate the mechanism through which FOXO1 regulates HRK expression and show that it involves inactivation of the HRK transcriptional repressor DREAM through a caspase-dependent process that is activated by transcriptional targets of FOXO1.

These findings are important because they provide further support for clinical testing of BCR signaling inhibitors not only in DLBCL, with chronic active BCR signaling, but also in DLBCL that is dependent on tonic BCR signaling. A crucial issue for future studies will be to determine what type of BCR signaling inhibitor would be most appropriate for patients belonging to each of the 2 DLBCL subsets with an activated BCR pathway. Based on the results of this study and the study of Chen et al,5 both SYK and PI3K inhibitors would be expected to be active in BCR-dependent DLBCL, independently of the mechanism that activates the BCR pathway. Although clinical data are scarce, the SYK inhibitor fostamatinib, which is the prodrug of R406, has already shown clinical activity in patients with DLBCL, with 22% (5/23) of the treated patients responding to treatment.6 A similar response rate was observed in the phase 1/2 clinical trial with ibrutinib in patients with relapsed/refractory DLBCL, with 25% (20/80) of patients responding to treatment.4 Interestingly, however, no patient with DLBCL responded to treatment with the selective PI3Kδ inhibitor idelalisib in the phase 1 study of relapsed/refractory non-Hodgkin lymphoma.7 The lack of responses in that study is probably a result of the small number of enrolled DLBCL patients (n = 9), but may also indicate that targeting PI3Kδ is insufficient to completely block the tonic BCR signal. This possibility needs to be considered in view of findings in murine models suggesting that the tonic BCR signal can be mediated by either PI3Kα or PI3Kδ, whereas antigen-dependent signaling is mediated only by PI3Kδ.8,9 Moreover, clinical responses have been observed in DLBCL patients with the dual PI3K-δ/PI3K-α inhibitor BAY 80–6946,10 indicating that both PI3K isoforms could be involved in transducing BCR signals in DLBCL.

Another important question that still remains unresolved is how to identify DLBCL patients who are most likely to respond to treatment with BCR signaling inhibitors. The use of the ABC DLBCL gene-expression signature has been proposed as a biomarker to enrich for patients with chronic active BCR signaling that are more likely to be sensitive to ibrutinib treatment, based on the higher response rate in ABC than GCB DLBCL (37% vs 5%, respectively).4 In the study by Szydłowski et al, expression of FOXO1, which was absent in 20% of the tumors, was proposed as a potential biomarker to identify patients who should be resistant to SYK or PI3K/AKT inhibitors. Although these biomarkers are undoubtedly valuable, the majority of patients that they would select would still be expected to be unresponsive to treatment. Therefore, further efforts to identify biomarkers for tonic and chronic BCR pathway activation will be essential for the development of personalized therapies with BCR signaling inhibitors in DLBCL.

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**REFERENCES**


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**MYELOID NEOPLASIA**

Comment on Garcia-Montero et al, page 761

**Origin of stem cells in the BM niche: new clues from mastocytosis**

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In this issue of Blood, Garcia-Montero et al1 reported that in nearly 30% of patients with indolent systemic mastocytosis (SM), the characteristic c-kit mutation D816V,2 is not restricted to mast cells, but may also be found in bone marrow tissue.
(BM)-derived mesenchymal stem cells (MSCs), also known as BM stromal cells (BMSCs). Thus, the notion that mastocytosis is a disease driven exclusively by mutations in blood cells, especially mast cells, has now been challenged.

Shortly before Garcia-Montero et al published their observations, we reported that clonal populations of MSCs from mastocytosis patients have impaired skeletal stem cell and hematopoietic support properties. We argued that dysfunctional mast cell/MSC interactions may drive epigenetic changes and pathological behavior in the MSC population. Based on Garcia-Montero’s findings, this may be true in the majority of patients, but the authors have taken the story one step further. When they analyzed a significantly larger number of fresh BM samples from patients with SM than we did, they detected c-kit mutations in highly purified, early-passage MSCs in 1 of 4 patients. Although quantitative polymerase chain reaction (PCR) experiments were performed with appropriate controls, it is remotely possible that these cultures were still contaminated with small numbers of mast cells. In future experiments, one could eliminate this possibility by analyzing clones of cells derived from single MSCs or with single-cell PCR, but for now, it is interesting and instructive to take the authors’ conclusions at face value. They raise several important questions regarding the pathophysiology of mastocytosis as well as the possible connection between the developmental origin of skeletal stem cells and hematopoietic stem cells. There are now a number of examples of BMSC-driven or dependent pathologies in the BM niche in myelodysplastic syndromes. Recent reports suggest that skeletal stem cells and hematopoietic cells in the BM cavity may have reciprocal interactions. BMSCs create a 3-dimensional network, and by secreting various growth factors and other signaling molecules, they play a central role in controlling the BM microenvironment in health and disease. How a c-kit mutation affects this regulatory function remains to be determined, and the difference between mutant BMSCs and wild-type BMSCs in mastocytosis patients should be explored.

In which kind of cell does the D816V mutation arise? The following possibilities should be considered (see figure). First, HSCs and BMSCs may be derived from a common stem/progenitor cell. If a mutation were to take place in this hypothetical stem cell, both hematopoietic and the mesenchymal cells would be expected to harbor the genetic defect. Although the existence of such “super” stem cells in the adult hematopoietic niche continues to be debated, there is a plausible candidate—the very small embryonic-like stem cells. These cells appear to have a differentiation potential that goes beyond the well-established lineage restriction of HSCs and BMSCs.

Another possibility is that the mutation arises in a hematopoietic cell progenitor that can give rise to stromal cells. This may be a process that is analogous to the creation of myeloid-derived fibrocytes, many of which eventually stop expressing CD45, obscuring their hematopoietic cell origin. Although reports suggesting that cells might arise in this way are few in number, there is evidence that this sort of transformation could occur.

Those scientists who are averse to the idea that mast cells and BMSCs might have a common precursor are left with a somewhat unattractive alternative—that c-kit mutations in the 2 cell populations occurred independently. If the putative mutation takes place in a BMSC progenitor, most sorts of BMSCs would be expected to have it. If it occurred in a more differentiated cell, the resulting clone might have a more narrow set of functions and resemble skeletal stem cells, pericytes, CAR cells, stromal fibroblasts, etc. It would certainly be interesting and important to study clonally selected mutant MSCs in more detail.

The existence of such cells would raise another possibility—that BMSCs could have gain-of-function mutations in diseases that share some similarities with mastocytosis, but lack mast cell pathology. Again, analysis of single cell-derived BMSC colonies or single-cell PCR studies of fresh uncultured BM-derived MSCs could readily address the questions listed here. The methods available to us have become so powerful that we should no longer be constrained by dogma. Rather than debating hypotheses like the ones we have outlined in this commentary, we should simply test them. It would be exciting to rewrite our textbooks.

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REFERENCES


6. Low JH, Ramdas P, Radhakrishnan AK. Modulatory effects of mesenchymal stem cells on leucocytes and
Comment on Aghourian et al, page 769

Gas6 fueling tumor-mediated thrombosis

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In this issue of Blood, Aghourian et al demonstrate that growth arrest-specific protein 6 (Gas6) contributes to tumor-induced venous thromboembolism (VTE) by promoting the expression of platelet-activating prostaglandin E2 (PGE2) in the endothelium.1

VTE is a well-recognized complication of different malignancies and substantially contributes to cancer-associated mortality. Understanding how different tumors modulate hemostasis is of the utmost importance for the development of next generation anticancer therapeutics, as well as for modulating current treatment regimes (see later in text). The occurrence of cancer-associated VTE is explained partially by the ability of tumors themselves to promote a procoagulant milieu both locally and systemically. For example, tumors may express tissue factor (TF) themselves or secrete procoagulant tissue factor-bearing microvesicles and release proinflammatory cytokines and proteases, resulting in activation of the coagulation cascade and downregulation of anticoagulant pathways.2

Aghourian et al unravel a novel link between tumor-mediated thrombosis and Gas6, which may provide yet another explanation for the procoagulant phenotype of many tumors. Gas6 is a vitamin K-dependent ligand for the receptor tyrosine kinase family comprising Tyro3, Axl, and Mer (TAM). It has the ability to bind to negatively charged phospholipids, such as phosphatidylserine exposed on apoptotic cells, which allows Gas6 to bridge TAM-expressing phagocytes to apoptotic cells thus potentiating phagocytosis. The Gas6-TAM system is a crucial anti-inflammatory signaling complex in immune cells, in addition to which it acts as a prosurvival factor, not only in tumors but also in the endothelium. Despite being homologous to the anticoagulant protein S, Gas6 has been shown to act mainly as a procoagulant factor. Gas6−/− mice are protected against lethal venous thrombosis, explained by a weakened platelet aggregation response3 and by decreased tissue factor expression in the endothelium.4 However, as the in vitro findings regarding platelet responses are of much milder character than what could be expected based on the striking phenotype of Gas6−/− mice, it is likely that yet unknown functions of Gas6 in regulation of thrombosis will emerge.

The work of Aghourian et al establishes a novel prothrombotic function of Gas6 independent of endothelial tissue factor regulation. In this study, the authors investigated changes in protein expression patterns on coculturing of endothelial cells from wild-type (WT) and Gas6−/− mice with M27 lung cell carcinoma. They identified the prostaglandin E synthase (Ptges) as a target protein upregulated in endothelium from WT mice but not in endothelium from Gas6−/− mice. Ptges is induced under proinflammatory stimuli, such as on exposure to cytokines, and the authors speculate that tumor-secreted cytokines or tumor-derived microvesicles might drive the increased endothelial Ptges expression. In a FeCl3-induced venous thrombosis model in either WT or Gas6−/− mice challenged with M27 cells or a B-cell lymphoma, the authors show that Gas6 deficiency protects the mice from a tumor-mediated increase in thrombus size. In Gas6-expressing endothelium, tumor cells were able to induce extracellular signal-regulated kinase (Erk)1/2 activation, resulting in downstream upregulation of Ptges and subsequent production and release of PGE2.
Origin of stem cells in the BM niche: new clues from mastocytosis

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