specific agents ex vivo or other related experiments using this model could possibly distinguish one mechanism from the other.

The development of heterogenous AML (and LSC) models allows characterization of corresponding molecular changes that mediate disease relapse and aggressiveness. The correlation between STAT hyperactivation and AML has been well documented.3,4 In the different 2-oncogene models (MN1 + ND13 or MN1 + HOXA4), Heuser et al found that cell growth was stimulated by GM-CSF, which, in turn, induced hyperphosphorylation of Stat5 and Stat1. To examine the requirement of these 2 genes in LSC expansion and proliferation, genetic models (Stat1−/− and Stat5−/− mice) were used. Stat5b deficiency in the 2-oncogene-expressing cells (MN1 + HOXA4), and to a lesser extent Stat1 deficiency, suppressed both GM-CSF–induced growth proliferation in vitro and LIC frequency in vivo. Thus, Stat5 up-regulation appears to enhance LSC self-renewal and provides further rationale for utilization of STAT5 inhibitors in treatment of human AML, as has been proposed by a variety of groups. It should be noted that although LIC frequency and proliferation of the 2-oncogene–expressing cells was significantly reduced in Stat5−/− mice, the cells remained leukemogenic, demonstrating that targeting of the STAT5 pathway will likely be insufficient to treat AML. Clearly, in light of the recently reported role of Stat5 in maintaining quiescence of normal hematopoietic stem cells,5 trials of such agents should be attended by careful companion studies seeking to validate effects on the target cell population and the nontarget population as well.

Murine models of AML (and human malignancies in general) have been limited by problems: either the genetic alterations used to initiate the malignancies in mice are not commonly found in human cancers or inactivation of homologs of human cancer-causing genes in mice does not replicate the disease phenotype. However, in this manuscript, the 2-oncogene model is indeed relevant to human AML. The group found that these same 2 oncogenes (MN1 and HOXA4) were concomitantly altered in the most aggressive and most cyogenetically unstable human AMLs and also linked this subset of AML to the highest levels of STAT activation. The group has developed an important and relevant model of poor-prognosis AML. It should ultimately allow direct testing of rationally designed agents that will effectively target LSCs without negatively influencing nonleukemic stem cells and progenitors.

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

REFERENCES


To explore mechanisms associated with effects of MAL on proplatelet formation, the authors examined gene expression, which showed a decrease in the levels of MMP-9 and MYL9 expression in MAL–down-regulated cells. Luciferase assays in HEK293T cells and chromatin immunoprecipitation in primary megakaryocytes demonstrated that the MAL/SRF complex directly regulates myosin light chain 9 (MLC9) and MMP-9 in vitro. The authors focused on these 2 factors, among others regulated by acute myeloid leukemia (AML), because of their known effects on cell migration. Indeed, megakaryocytes migration in response to SDF-1 was decreased following MAL knockdown, potentially implicating MMP-9 as a mediator of the effect of MAL on migration. Phosphorylation of MYL9 has already been reported to activate MYH9 and to negatively regulate proplatelet formation in normal MK.8 Here, Gilles et al showed by application of MYL9 shRNA that MYL9 is involved in proplatelet formation.

In the future, it would be interesting to address differences between mouse and human models, in light of the thrombocytopenia
and increase in proplatelet-forming megakaryocytes seen in MYH9-deficient mice. Also, the authors do not rule out the possibility of involvement in proplatelet formation of other genes identified as regulated by MAL. For instance, the focus in the study at hand has been only on displayed genes known to have a key role in cytoskeleton organization. Future studies could explore the functional significance for proplatelet formation of other genes that are deregulated as a result of MAL down-regulation or overexpression. Finally, also of interest is the possibility that alpha granules heterogeneously scattered in the cytoplasm of AML-deregulated cells bear heterogeneous contents, a phenomenon that has been recently explored in other studies.

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*THROMBOSIS & HEMOSTASIS*

Comment on Undas et al, page 4272

**Denser matters**

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In this issue of *Blood*, Undas and colleagues show that the structure of the blood clot is altered in patients with venous thrombosis and their relatives compared with healthy controls.1

It was known from previous studies that the structure of the blood clot is different in patients with coronary artery disease3 and their relatives.5 The findings presented in this issue by Undas et al contribute new and important lines of evidence, showing that blood clot structure may play a role in mechanisms of thrombosis. First, the clot characteristics found in the patients with venous thrombosis are similar to those found in patients with arterial thrombosis. They have a denser structure and smaller pores, suggesting that clot structure plays a role in thrombosis in both types of vasculature. Second, in agreement with previous findings in relatives of patients with coronary artery disease6 and peripheral arterial disease,7 the relatives of patients with venous thrombosis show clot structure characteristics that are intermediate between those of the patients and the controls, suggesting that genetic or common environmental factors play a role. Finally, the authors found that clot structure of patients with a complicating pulmonary embolism show different features compared with patients with deep vein thrombosis only, indicating a potential role of clot structure in the fragmentation of the thrombus.

It is tempting to speculate on how clot structure may contribute to thrombosis, but some caution is required until more is known about the mechanisms involved. The clot is composed mainly of polymerised fibrin, which is formed when thrombin cleaves fibrinopeptides A and B from fibrinogen. Other proteins and blood cells interact with the fibrin to generate a final blood clot. Clots generated in the arterial circulation are rich in platelets, while clots from the venous circulation are fibrin and erythrocyte rich. Flow has been shown to affect structure of the clot and to produce areas of different architecture in the blood clot.8,9 It should be noted that in the current study, as was the case with most previous studies, clot structure was investigated ex vivo in the absence of flow and cells. Even so, the finding that similar features of fibrin clot structure associate with thrombosis in both the venous and arterial circulation indicates the existence of a common mechanism by which fibrin structure influences risk for thrombosis regardless of the effects of flow and cells.

Two possible mechanisms by which clot structure may influence the risk for thrombosis stand out from others. One of these involves the response of the clot to endogenous fibrinolytic mechanisms. The structure of the fibrin clot is a major determinant of the efficacy of fibrinolysis. Indeed, Undas et al show that the clots from patients with venous thrombosis are lysed more slowly than those of their relatives, which in turn lyse more slowly than those of the controls.4 This is in complete agreement with the structural changes that were observed in these subjects. The second potential mechanism involves the response of the clot to mechanical deformation and stress induced by the flow of the blood. Fibrin is one of the most elastic natural polymers known to man,7,8 The architecture of the fibrin network modulates visco-elastic properties of the clot. Differences in clot structure and elasticity could therefore influence the response of the clot to shear stress and flow, and possibly the risk of embolization. To investigate this, the authors used compaction as an estimate of clot stiffness and observed changes in the patients that correlate with the structural parameters of the fibrin clot.1 Fibrin structure and compaction were different in the patients with pulmonary embolism, although counterintuitively perhaps they were intermediate to fibrin structure and compaction in the patients with deep vein thrombosis alone and their relatives. Nonetheless, these findings indicate that “brittleness” of the clots may play a role in thromboembolism and suggest that further research is required to fully characterize changes in visco-elastic properties of blood clots.

The implications of the findings presented by Undas et al are that the structure and function of the blood clot are important for thrombosis. Once the molecular mechanisms that underpin the relationship between clot structure and thrombosis have been fully delineated, consideration should be given to the diagnostic potential of characterizing clot structure and to the modulation of clot architecture as a possible treatment for thrombosis.

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MAL: not just a leukemia inducer

Katya Ravid