site located within the HD-LNR complex, and that displacement from the cell surface makes this sequence more accessible for protease cleavage" (page 739; see figure panel A). In conclusion, the authors have identified a novel family of NOTCH1-activating mutations that result in aberrant levels of ICN1, further expanding our understanding of the important molecular parameters that control Notch activity.

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

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NEOPLASIA

Comment on Ellis et al, page 741

Taking a SNPshot of t-AML

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In this issue of *Blood*, Ellis and colleagues report on the interaction of SNPs in the p53 tumor suppressor pathway and in the *MDM2* 309 locus in susceptibility to therapy-related AML.

Genetic variants are associated with disease susceptibility.[1,2] Of the genetic variants, single-nucleotide polymorphisms (SNPs) lend themselves to interrogation in population-based studies. As reported in this issue of *Blood*, Ellis and colleagues studied 2 separate, large cohorts of therapy-related acute myeloid leukemia (t-AML) patients. Because only a subset of all patients treated with cytotoxic chemotherapy or radiation develop t-AML, it was hypothesized that these individuals may be predisposed due to constitutional genetic variations in DNA damage-response pathways. Although several candidate genes have been previously implicated, Ellis et al focused on the p53 tumor suppressor pathway, as this transcription factor mediates cell-cycle arrest, cell senescence, and apoptosis, and is often lost or mutated in t-AML. They also examined a common SNP (SNP309) of MDM2, a ubiquitin E3 ligase, which negatively affects the stability of p53 and has been examined previously in other series of leukemia patients.[3] As reported in their article, an arginine (Arg) at *TP53* codon 72 predisposes cells to apoptosis, whereas a proline (Pro) mediates cell-cycle arrest. At the *MDM2* SNP309, a G allele indicates high binding ability of SP1 transcription factor, which increases levels of MDM2, thereby decreasing p53 expression. In contrast, a T allele allows increased p53 function.

The cohort of t-AML patients from the University of Chicago was selected because of availability of Epstein Barr virus–transformed lymphoid lines from which DNA could be extracted, whereas peripheral blood DNA was available from the cohort of patients studied from the United Kingdom. A total of 171 cases were studied. It was found that neither *p53* nor *MDM2* variants by themselves were associated with t-AML risk, but there was an interaction that influenced susceptibility. The figure illustrates the models proposed for these interactive influences. Control cohorts were used to determine that there was not a bias in baseline frequencies of the SNPs, and this SNP interaction was not observed in de novo AML cases. The same interactive influence was noted in those treated with chemotherapy and those who acquired abnormalities of chromosomes 5 or 7. Only *TP53* Pro/Pro was associated with increased risk of t-AML in those who received chemotherapy alone. No significant effects on disease latency were noted. The *MDM2* TT genotype appeared to offer a protective effect in younger women.

Although this study used 2 relatively large cohorts of t-AML patients, it would have benefitted from the melding of differing methodologies and DNA sources (although these were well-controlled for to reduce bias, and genotype distributions appeared comparable between University of Chicago and United Kingdom control subjects). The 2 series used different means of case selection/identification and different treatment regimens. Also, given that multiple therapeutic regimens were utilized, the effect of SNP interaction as related to exposure to a single agent or combination regimen on development of t-AML could not be determined. Nonetheless, this study demonstrates that interrogating biologically rational interactions between SNPs may be important in determining the risk of susceptibility to disease. Such interactions might also influence the clinical course of disease or define genetic variations that predict different toxicities and efficacies of available treatments.

The 2 SNPs examined in this work are no doubt only a snapshot of the total picture of susceptibility to therapy-related AML, but studies such as this are a beginning to improve our understanding of genetic susceptibilities. If confirmed in prospectively analyzed cohorts or other large retrospective cohorts of t-AML, these markers of therapy-related AML susceptibility might

Model 1	*MDM2* 309 TT	and	*TP53* 72 Arg/Arg	Increased t-AML risk
		OR		
	MDM2 309 GT or GG	and	*TP53* 72 Pro/Arg or Pro/Pro	
Model 2	*MDM2* 309 TT	and	*TP53* 72 Pro/Arg or Pro/Pro	Decreased t-AML risk

MDM2 and TP53 interaction in t-AML. Data regarding interactions between *MDM2* 309 and *TP53* 72 alleles were consistent with double-homozygous-state TT and Arg/Arg, or any genotype with at least one *MDM2* SNP 309G and one *TP53* codon 72 Pro resulting in increased risk of t-AML. Any *TP53* Pro–containing genotype with *MDM2*TT was protective.

influence the choice of therapeutics to treat malignancies for which alternate therapies are available. They also provide preliminary insights into mechanisms of leukemogenesis, which may facilitate development of targeted therapies for t-AML.

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

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RED CELLS

Comment on Patel et al, page 856

It really *IS* the red cell

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In this issue of *Blood*, Patel and colleagues demonstrate that placental growth factor derived from hemoglobin S erythroid cells upregulates the expression of both ET-1 and ET-BR via HIF-1α in the absence of hypoxia.

It has become increasingly clear that the process leading to vaso-occlusion in sickle cell disease (SCD) is quite complex and likely brings into play not only red-cell adhesion and red-cell sickling, but also leukocyte adhesion and activation, cytokine production, activation of coagulation, and induction of endothelial-cell activation. Together, these processes lead to further exacerbation of the occlusive process, hypoxia reperfusion injury, and extension of tissue damage. Thus, many investigators have focused recently on the role played by the nonerythroid cells and factors involved in these processes. Attention has been paid to the role of leukocytes, the elevated levels of proinflammatory cytokines, the activation of thrombogenesis, and platelet activation. And yet, one must ask: do all these cells and processes become involved just because sickled red cells become stuck in small vessels?

Patel and colleagues in this issue of *Blood* now redirect our attention to the primary cause of sickle cell disease and vaso-occlusion—the red blood cell. Sickle cell disease is, after all, a disease of hemoglobin (Hb), whose expression is restricted to red blood cells. While the propensity of cells with predominantly Hb S to sickle at low oxygen tension and to adhere to endothelial cells was described decades ago, the degree to which abnormal red cells play a truly dynamic role in sickle cell disease is only now being appreciated.

Connecting the dots, Patel and colleagues have now discovered an intriguing pathway that may explain a great deal, especially about the process of lung and pulmonary vascular injury in SCD. Tordjman et al showed in 2001 that erythroid cells were the only bone marrow hematopoietic cells that coexpressed 2 angiogenic factors, VEGF-A and PlGF; moreover, they showed that expression of these factors increased during erythroid maturation, and that erythroblasts secreted these factors and thus were capable of inducing migration of both monocytes and endothelial cells.[1] PlGF is a member of the vascular endothelial growth factor (VEGF) family of proteins. It is typically secreted and interacts with several receptor tyrosine kinases in the VEGFR family. In addition to being a proangiogenic factor, it is also proinflammatory and may play an important role in the instability of atherosclerotic plaques, as well as in tumor neovascularization.

In 2003, companion papers by Perelman et al[2] and Selvaraj et al[3] showed that levels of PlGF were increased in SCD at least roughly proportionately to the frequency of vaso-occlusive episodes. They also showed that PlGF directly activated monocyte chemotaxis and mRNA levels of interleukin-1, interleukin-8, monocyte chemoattractant protein-1, and VEGF. Furthermore, Hb SS erythroid cells appear to contain more PlGF per cell than do normal cells, and this is hypothesized to account for the increased PlGF levels in SCD.[4] Investigation of the mechanism whereby PlGF stimulated monocytes revealed that PlGF activates the monocyte Flt-1, which then leads to activation of PI2 kinase/AKT and ERK-1/2 signaling.[4]

In their paper in this issue of *Blood,* Patel and colleagues have now shown that the pathways activated by PlGF are potentially involved in the development of SCD-associated pulmonary hypertension, a grave complication affecting approximately one-third of adults with SCD. They showed not only that PlGF induces increased expression of endothelin-B receptor (ET-BR) by monocytes, but also that it induces expression of endothelin-1 (ET-1), an ET-BR ligand, by human microvascular endothelial cells. ET-1 is known to be increased in SCD and to become further elevated during vaso-occlusive episodes and acute chest syndrome.[4] Interestingly, the effects of PlGF on endothelial cells and monocytes occurred via activation of PI-3 kinase and also involved hypoxia-inducible factor-1α (HIF-1α) in the absence of hypoxia. These effects potentially constitute a double whammy that may lead to a vicious cycle of both vasoconstriction and inflammation in the pulmonary circulation.

At this point, definite links between PlGF-induced processes and pulmonary hypertension have not been established. For example, serum PlGF levels during pregnancy peak at

Table 1. Characteristics of the pathogenic sickle red cell

Red cell characteristic	Effects
Cell dehydration	Increased dynamic rigidity; increased hemoglobin polymer formation and sickling; increased blood viscosity
Hemoglobin polymer formation	Sickle shape; mechanical obstruction of small-caliber vessels; hemolysis; vaso-occlusion
Young age	Increased expression of adhesion receptors; increased content of signaling molecules; activation of adhesion receptors
Surface phosphatidylserine exposure	Thrombogenic potential; activation of coagulation cascade; adhesion
Adhesive properties	Abnormal interactions with other blood cells (monocytes, neutrophils, platelets) and endothelium; vaso-occlusion; inflammation
Oxidatively damaged membrane	Defect in NO transport and delivery; abnormal cell rheology; vasoconstriction; inflammation
Abnormal cell-cell signaling	Activation of endothelial cells and monocytes; inflammation; vasoconstriction