GM-CSF signaling was likely suppressing RUNX1-ETO leukemogenesis by enhancing differentiation of preleukemic myeloid progenitor cells.

Although these results highlight a novel role of GM-CSF signaling in tumor suppression, the conclusions are limited in part by the expression of RUNX1-ETO on a βc-null background, which does not accurately mimic GM-CSFRα haploinsufficiency observed with sex chromosome loss. Given the median survival of 230 days when RUNX1-ETO was expressed on the βc-null background, it is likely that the authors would not have observed AML progression in the lifespan of βc heterozygous mice, although these experiments remain to be done. These data are also interesting from the perspective that GM-CSF signaling is known to stimulate both survival and proliferation of myeloid progenitor cells, with lower concentrations of GM-CSF able to enhance survival. It might therefore have been expected that activating alleles of βc, and not βc loss, would cooperate with RUNX1-ETO in leukemogenesis. On the contrary, the results may explain why activating mutations in GM-CSFRα or βc have not been noted in t(8;21)+ patient samples because these mutations might function to enhance myeloid differentiation and/or inhibit progenitor cell self-renewal. These results also raise the cautionary note that efforts aimed to inhibit signaling by IL-3 or GM-CSF in treatment of AML may actually be leukemia-promoting in some clinical contexts, specifically in t(8;21)+ AML.

A lingering question is whether Matsuura and colleagues have uncovered the major factor explaining the selective advantage for sex-chromosome loss in t(8;21)+ AML. Given that the whole sex chromosome is typically lost and not the individual CSF2RA locus, it is likely that additional factors are acting to enhance RUNX1-ETO-associated leukemogenesis when haploinsufficient on the sex chromosome. If GM-CSFRα loss were sufficient, then it would also have been anticipated that t(8;21)+ patient samples might have common deletions of the βc locus on chromosome 22, which has not been observed, or frequent deletions of the closely linked IL-3–GM-CSF–IL-5 cytokine gene cluster on human 5q, which is deleted on 1 allele in some AML and myelodysplastic syndrome cases but not commonly absent in RUNX1-ETO+ samples. These observations suggest that the important and challenging hunt for the mechanism explaining frequent sex chromosome loss in t(8;21)+ leukemias will likely continue.

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Comment on Dayal et al, page 3176

Homocysteine and thrombosis: guilt by association?

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The long-recognized connection between homocysteine and thrombosis is examined in this issue of Blood in a study conducted by Dayal and colleagues. The results challenge the proposed mechanisms by which disordered homocysteine metabolism triggers vascular disease.

Homocysteine is a structural intermediate generated during the synthesis of cysteine from methionine. In addition to being metabolized to cysteine via a transulfuration pathway, homocysteine can be processed back to methionine via a remethylation pathway.

Abnormal homocysteine metabolism is linked to vascular disease, including endothelial dysfunction, but is hyperhomocysteinemia sufficient to trigger thrombosis? Key components of the homocysteine pathway are shown. THF indicates tetrahydrofolate; MTHFR, methylene tetrahydrofolate reductase; and CBS, cystathionine-β-synthase.
The enzymes regulating both pathways require B vitamins (B6, B12, and folic acid) as cofactors (see figure). Normal plasma levels of homocysteine are ~5–15 μM. Epidemiologic studies have shown that elevated plasma levels of homocysteine are independently associated with cardiovascular diseases, including venous thromboembolism and atherosclerotic arterial diseases, such as myocardial infarction and stroke. A variety of factors can produce hyperhomocysteinemia. In humans, severe hyperhomocysteinemia with associated homocystinuria is most commonly due to homozygous deficiency of cystathionine-β-synthase (CBS). This rare genetic disorder, in which plasma homocysteine levels typically exceed 100 μM, is characterized by venous thromboembolism, premature atherosclerosis, osteoporosis, and developmental and ocular abnormalities. In contrast, mild-moderate elevation of plasma homocysteine (ie, 15–50 μM) is not uncommon, occurring in 5% to 7% of individuals. Modest elevations of plasma homocysteine are independently associated with an increased incidence of venous thrombosis and arterial atherothrombotic diseases, including myocardial infarction and stroke. The most common genetic cause of mild hyperhomocysteinemia is the expression of a thermolabile variant of methylene tetrahydrofolate reductase (MTHFR), which results in inhibition of remethylation of homocysteine back to methionine. Nutritional deficiencies of vitamins B6, B12, and folic acid can also produce mild-moderate elevations of plasma homocysteine. Some medications and diseases, including kidney disease and hypothyroidism, can also induce hyperhomocysteinemia. Mechanistic studies have shown that homocysteine can exert a variety of effects expected to promote thrombosis and cardiovascular disease, including increased expression of adhesion molecules, cytokines, tissue factor, and blood coagulation factor V, inhibition of fibrinolysis, disruption of nitric oxide metabolism, and increased platelet reactivity.

Given the existing epidemiologic and mechanistic data, it has generally been believed that homocysteine is prothrombotic. Remarkably, several clinical trials have shown that B vitamin replacement therapy, which effectively lowers plasma homocysteine concentration in patients with moderate homocysteinemia, does not lower cardiovascular risk. Furthermore, in patients with severe hyperhomocysteinemia because of CBS deficiency, vitamin B therapy in conjunction with a low methionine diet significantly improves vascular outcomes despite leaving most individuals with a residual of moderately elevated plasma homocysteine levels, suggesting that downstream targets other than homocysteine might be mediating the beneficial effect of therapy.

So does elevated plasma homocysteine cause thrombosis and vascular disease? Experimentally induced elevated plasma homocysteine concentration has been shown to induce endothelial dysfunction in rodents and monkeys. To examine the role of hyperhomocysteinemia in thrombosis, Dayal and colleagues studied mice with complete deficiency of CBS that also expressed a transgene encoding a mutant form of human CBS that rescued the neonatal lethality associated with complete CBS deficiency, yet left mice with severe hyperhomocysteinemia (mean plasma concentration 245 μM). Despite this severe metabolic disturbance, hyperhomocysteinemic mice did not exhibit any tendency toward accelerated thrombosis; in fact, they demonstrated significantly delayed thrombosis compared with controls in one of the experimental models employed. To their credit, Dayal and colleagues used multiple experimental models to characterize the mice, including different forms of arterial injury and a model of venous thrombosis. They also gave all mice the same diet, thereby avoiding possible confounding effects of altered diet on thrombosis end points, which had been a limitation of prior studies that found that mice with diet-induced hyperhomocysteinemia exhibited accelerated thrombosis.

What can we conclude from the negative results reported by Dayal and colleagues? As a whole they suggest that severe hyperhomocysteinemia, in itself, is not sufficient to promote thrombosis. Rather, other factors associated with abnormal homocysteine metabolism may be responsible for the propensity to thrombosis observed in individuals with severe hyperhomocysteinemia. One such candidate is hypermethioninemia, which is associated with hyperhomocysteinemia. While the authors’ data did not support this hypothesis, it is difficult to examine this issue in the genetic model that was employed. Of course, it is difficult to prove a negative. As the authors acknowledge, mice and humans may differ significantly in the expression and/or function of potential prothrombotic factors downstream of homocysteine, which could explain the lack of a prothrombotic phenotype in the mice that were studied. Future studies involving large-animal models of thrombosis could prove useful in addressing this issue. However, for the time being, the best available models of hyperhomocysteinemia appear to be murine. From the clinical perspective, the study by Dayal and colleagues is intriguing in that it helps to explain an existing paradox in the field, that is, while hyperhomocysteinemia is independently associated with increased risk of thrombosis, pharmacologic strategies that effectively lower plasma homocysteine concentration do not reduce the risk of thrombotic vascular events.

Where do we go from here? Dayal and colleagues point the field toward further studies of the molecular and cellular mechanisms underpinning the connection between disordered homocysteine metabolism and thrombosis. Hopefully such studies will identify the factor(s) that function independently of homocysteine, or in concert with it, to promote thrombosis, and eventually lead to the development of effective strategies to improve vascular outcomes in patients with hyperhomocysteinemia.

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