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Comment on Matsuura et al, page 3155

# GM-CSFRα: the sex-chromosome link to t(8;21)$^+$ AML?

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In this issue of *Blood*, Matsuura and colleagues provide evidence that loss of GM-CSF signaling promotes leukemic progression in association with one of the most frequently observed cytogenetic abnormalities in AML, the t(8;21)(q22;q22) that generates the RUNX1-ETO fusion protein.[1]

In the early 1970s, Janet Rowley's group discovered the first recurrent cytogenetic abnormality in acute myeloid leukemia (AML), which involved translocation of the *RUNX1* gene on chromosome 21 to the *ETO* gene on chromosome 8.[2] The t(8;21)(q22;q22), which is present in 8% to 10% of AML cases, results in an in-frame fusion protein, RUNX1-ETO, that has been shown by animal modeling studies to be necessary but not sufficient for leukemogenesis. When expressed from either the endogenous *Runx1* locus in mice or from various transgenes as the sole mutation, RUNX1-ETO primarily functioned to enhance hematopoietic stem/progenitor cell self-renewal, slowed progenitor cell proliferation, and partially blocked myeloid differentiation resulting in a slow accumulation of myeloblasts/promyelocytes in bone marrow over the lifespan of the animal with rare progression to AML. The suppression of proliferation by RUNX1-ETO suggested that changes leading to leukemic progression would likely be in pathways regulating cellular proliferation, which has been supported by analysis of t(8;21)$^+$ patient samples where approximately 70% of cases have non-overlapping mutations resulting in constitutive activation of receptor tyrosine kinases (RTKs), particularly c-KIT, and activating mutations in downstream signaling molecules like NRAS or KRAS. Animal modeling studies where activating RTK mutations are coexpressed with RUNX1-ETO have shown cooperation in acceleration of AML progression, but other changes are necessary to stimulate an aggressive acute leukemia with short disease latency.

The search for additional AML-promoting factors that function in conjunction with RUNX1-ETO and RTK/RAS mutations, and characterization of the "missing" oncogenic pathways that are being contributed by these factors, has again been informed by analysis of t(8;21)$^+$ patient samples. The 2 most common recurrent cytogenetic abnormalities associated with t(8;21) include loss of a sex chromosome in a disproportionally high percentage of cases (50%–60%) and del(9)(q22) in 15% to 25% of samples.[3] Both the del(9q) and sex chromosome loss involve deletions of multiple genes and appear to involve haploinsufficiency as the major contributing factor to leukemogenesis given that deletions or inactivating mutations in genes within the deleted regions have not been found on the wild-type chromosomes.

Here, Matsuura et al have set out to identify the factor(s) on the human sex chromosomes that may be cooperating with RUNX1-ETO to promote AML. Because loss of either the X or Y chromosome is commonly observed in t(8;21)$^+$ patient samples, it has been proposed that haploinsufficiency must be occurring at loci within pseudoautosomal regions (PARs), which are shared sequences on the X and Y chromosomes that undergo pairing and recombination during meiosis.[4,5] Matsuura and colleagues begin their current work by showing that expression of RUNX1-ETO in XO bone marrow cells from *Patched fur* mutant mice, which have a high frequency of XO offspring because of X-Y nondisjunction in meiosis,[6] does not increase the incidence of AML compared with wild-type bone marrow cells expressing RUNX1-ETO. This observation led the authors to conclude that the critical gene(s) on human sex chromosomes might be localized to syntenic regions on murine autosomes.

One particularly interesting region on mouse chromosome 19 included the tightly linked loci encoding the GM-CSF receptor α (*CSF2RA*) and IL-3 receptor α (*IL3RA*) chains. GM-CSFRα, IL-3Rα, and IL-5Rα belong to the type I cytokine receptor family that function as low-affinity, ligand-binding chains that are converted to high-affinity receptors on association with the shared β common (βc) signaling chain. Inactivation of the βc locus in mice disrupts signaling stimulated by GM-CSF and IL-5 but IL-3 signaling remains intact because of a species-specific and functionally redundant homolog of βc, $\beta_{IL\text{-}3}$, which interacts specifically with IL-3.[7] Hematopoiesis in βc, or in βc/IL-3 double-mutant mice that completely lack GM-CSF, IL-3, and IL-5 signaling, is surprisingly normal with the exception of significant lung pathology and impaired immune responses including sensitivity to certain types of infections because of a severe reduction in eosinophils.[8]

To test whether loss of GM-CSF signaling (and IL-5 because of the approach) would promote progression to AML in conjunction with RUNX1-ETO, Matsuura et al used a bone marrow transduction/transplantation assay where RUNX1-ETO was expressed from a retroviral vector in βc-knockout bone marrow cells, which were then transplanted into irradiated wild-type recipient animals. Interestingly, loss of βc significantly accelerated progression to AML in association with RUNX1-ETO, with a relatively long median survival of 230 days. Hematopoiesis in animals reconstituted with βc-deficient cells transduced with a control vector was essentially normal. βc loss also shortened the time to AML using a more oncogenic derivative of RUNX1-ETO, RUNX1-ETO9a, indicating that loss of GM-CSF/IL-5 signaling was enhancing RUNX1-ETO–associated leukemogenesis. In further experiments, the authors showed that addition of GM-CSF, but not IL-5, inhibited the immortalization of RUNX1-ETO–expressing primary bone marrow cells in serial replating assays in vitro, suggesting that GM-CSF signaling was specifically suppressing RUNX1-ETO promotion of self-renewal. Results using murine cells were supported by an additional experiment using the human t(8;21)$^+$ Kasumi-1 cell line, which exhibits sex chromosome loss and a previously studied hyporesponsiveness to GM-CSF. Kasumi-1 cells transduced with either a retroviral vector expressing GM-CSFRα or the control vector were plated in methylcellulose in the presence of GM-CSF. Matsuura et al noted a 40% reduction in colony numbers specifically in cultures treated with GM-CSF, indicating that

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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.[9] 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.

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

## REFERENCES


1. Matsuura S, Yan M, Lo M-C, et al. Negative effects of GM-CSF signaling in a murine model of t(8;21)–induced leukemia. *Blood*. 2012;119(13):3155-3163.

2. Rowley JD. Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. *Annales de Genetique*. 1973;16(2):109-112.

3. Kuchenbauer F, Schnittger S, Look T, et al. Identification of additional cytogenetic and molecular genetic abnormalities in acute myeloid leukaemia with t(8;21)/AML1-ETO. *Br J Haematol*. 2006;134(6):616-619.

4. Brown MA, Harrison-Smith M, DeLuca E, et al. No evidence for GM-CSF receptor alpha chain gene mutation in AML-M2 leukemias which have lost a sex chromosome. *Leukemia*. 1994;8(10):1774-1779.

5. Simmler MC, Rouyer F, Vergnaud G, et al. Pseudoautosomal DNA sequences in the pairing region of the human sex chromosomes. *Nature*. 1985;317(6039):692-697.

6. Burgoyne PS, Evans EP. A high frequency of XO offspring from X(Paf)Y* male mice: evidence that the Paf mutation involves an inversion spanning the X PAR boundary. *Cytogenet Cell Genet*. 2000;91(1-4):57-61.

7. Nishinakamura R, Nakayama N, Hirabayashi Y, et al. Mice deficient for the IL-3/GM-CSF/IL-5 beta c receptor exhibit lung pathology and impaired immune response, while beta IL3 receptor-deficient mice are normal. *Immunity*. 1995;2(3):211-222.

8. Nishinakamura R, Miyajima A, Mee PJ, et al. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood*. 1996;88(7):2458-2464.

9. Guthridge MA, Powell JA, Barry EF, et al. Growth factor pleiotropy is controlled by a receptor Tyr/Ser motif that acts as a binary switch. *EMBO J*. 2006;25(3):479-489.


● ● ● THROMBOSIS & HEMOSTASIS

Comment on Dayal et al, page 3176

# Homocysteine and thrombosis: guilt by association?

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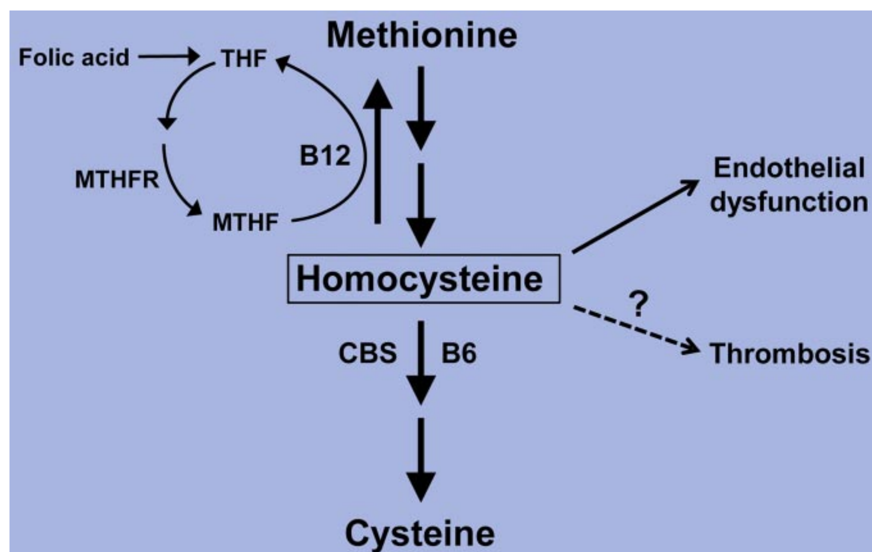
The long-recognized connection between homocysteine and thrombosis is examined in this issue of *Blood* in a study conducted by Dayal and colleagues. The results challenge the proposed mechanisms by which disordered homocysteine metabolism triggers vascular disease.[1]




**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.**

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.



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