

adhesion and activation. Therefore, the authors suggest that the need for physical contact between platelets and the subendothelium would explain the higher numbers of platelets required to prevent splenic necrosis than to provide systemic hemostasis. In this sense, the observation that platelets expressing integrin  $\beta 3$  and CD40L are required for LCMV clearance through CTLs<sup>5</sup> implies a physical interaction between platelets and immune cells and gives further support to the idea that more platelets are necessary to prevent splenic necrosis and viral replication than bleeding.

Loria et al also analyzed platelet involvement in the immunosuppression after LCMV clone-13 infection of mice by increasing the number of circulating platelets with thrombopoietin treatment. Even though the number of platelets was significantly increased, no differences were seen in LCMV viral titers, suggesting that other mechanisms mediate the deficient immune response seen in LCMV clone-13 infections.

From their present results, Loria et al propose interesting future lines of research, including an evaluation of the abilities of vasoactive molecules to prevent hemorrhage and death in experimentally platelet-depleted mice. They also propose that mice genetically deficient in thrombopoietin signaling, which have platelet levels similar to those found in humans, are a more appropriate experimental model for studying the pathology of VHF. They further suggest that the selective inactivation of IFN-I signaling in megakaryocytes, while preserving important IFN-I antiviral activity in immune and stromal cells, might completely overcome the thrombocytopenia induced by LCMV clone-13 infection. This interesting proposal could be more generalized as thrombocytopenia mediated by IFN-I might be involved in several viral infections.<sup>6</sup> Additionally and in line with this hypothesis, it was recently demonstrated that megakaryo-

cytes express functional IFN-I receptors.<sup>7</sup> Finally, an important issue that requires further clarification is the molecular basis governing platelet interaction with immune cells in VHF.

After being discovered in 1882 by Giulio Bizzozero, platelets were considered to be cytoplasmic “dust” derived from megakaryocytes. During the 20th century, enormous basic and clinical evidence entrenched platelets as critical mediators of physiologic hemostasis and pathologic thrombosis. In the present century, they are additionally appreciated as key amplifiers of the inflammatory response and, more recently, important regulators of the immune response. Along these lines, the study by Loria et al strongly supports the concept that, regardless of the apparent “simplicity” of platelets, these cells play critical roles in several cellular processes beyond hemostasis.

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

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Complete loss of chromosome 7 (monosomy 7) and partial deletion of the long arm of chromosome 7 [del(7q)] are recurring karyotypic abnormalities in malignant myeloid disorders, including the myelodysplastic syndromes (MDS) and AML, and are associated with a poor prognosis.<sup>2,3</sup> The del(7q) in MDS and AML is considered to mark the location for a gene or genes the loss of which may affect important processes such as growth control and normal hematopoiesis. The identification of significant genes associated with chromosome deletions, including the del(7q), in human leukemia has proven challenging, however.

The basis for research on deletions such as the del(7q) in MDS and AML is well known. The first step is to characterize the deletions and to identify the commonly deleted region (CDR), the region of deletion shared by all patients, as this localizes the gene(s) for further study. Over the years several CDRs mapping to 7q have been identified in MDS and AML, including CDRs at 7q22, 7q32-33, and 7q35-36.<sup>2-4</sup> The next step typically involves the sequencing of all the candidate genes that map within the CDR in a group of affected patients. The gene sequencing is critical to our understanding of the disease pathogenesis; if the Knudsen 2-hit model applies, there would be loss of 1 allele of a gene and a mutation of the remaining copy of the same gene. The lack of recurrent mutations identified in the genes mapping to the various CDRs identified on 7q in MDS and AML patients with  $-7/\text{del}(7q)$  suggests that haploinsufficiency, a dosage effect resulting from the loss of a single allele of a gene,<sup>5</sup> may be the molecular mechanism relevant in this group of malignancies. There has been growing recognition of haploinsufficiency as a cancer model over the past decade and the importance of this model in the context of myeloid disorders is supported by recent studies concerning MDS patients with the 5q- syndrome.<sup>6</sup>

Interest in the *CUX1* (*CUTL1*) gene, encoding a transcription factor, as a possible candidate gene in malignant myeloid disorders with abnormalities of chromosome 7, stretches back to the mid-1990s when it was first mapped to the CDR at 7q22 by investigators.<sup>2,7</sup> Most recently *CUX1*, normally highly expressed in multipotent hematopoietic progenitors, was shown to be expressed at reduced levels in CD34<sup>+</sup> cells from patients with MDS with  $-7/\text{del}(7q)$ .<sup>4</sup> McNerney and colleagues

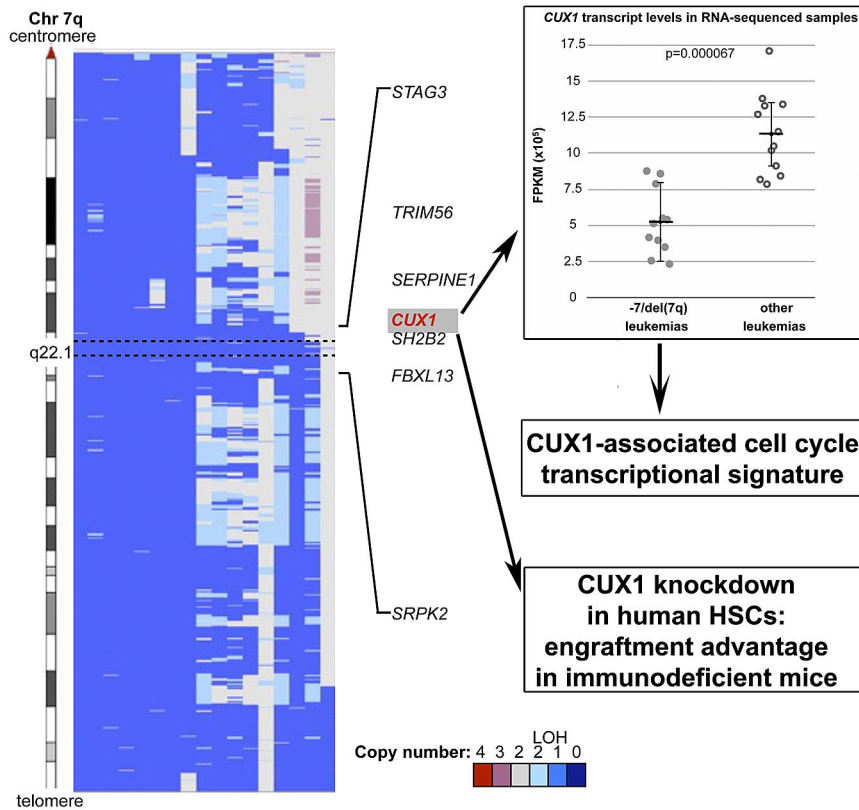
## ● ● ● MYELOID NEOPLASIA

Comment on McNerney et al, page 975

# CUX1 in leukemia: dosage matters

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In this issue of *Blood*, McNerney and colleagues identify *CUX1* as a tumor suppressor gene (TSG) on the long arm of chromosome 7, showing frequent inactivation in acute myeloid leukemia (AML).<sup>1</sup>



Copy number analysis of 7q derived from SNP arrays of leukemia samples with  $-7/\text{del}(7q)$  shows that *CUX1* maps within the 2.17 Mb CDR at 7q22.1. RNA-sequencing data showed that *CUX1* is expressed at haploinsufficient levels in  $-7/\text{del}(7q)$  leukemias and this is associated with a cell-cycle transcriptional gene signature. Haploinsufficiency of *CUX1* gave human hematopoietic cells a significant engraftment advantage upon transplantation into immunodeficient mice. Adapted from Figure 1 and Figure 3A in the article by McNerney et al that begins on page 975.<sup>1</sup>

have used SNP array analysis to refine the mapping of the CDR at 7q22, identifying a 2.17Mb CDR at 7q22.1 containing the *CUX1* gene in patients with de novo and therapy-related malignant myeloid disorders with  $-7/\text{del}(7q)$  (see figure).<sup>1</sup> Transcriptional profiling using a sensitive RNA sequencing method revealed that *CUX1* was haploinsufficient in this patient group and was, moreover, the most significantly differentially expressed transcript within the CDR (see figure).<sup>1</sup> Intriguingly, in 1 patient the *CUX1* gene was shown to be disrupted by a translocation, resulting in a loss-of-function RNA fusion transcript.<sup>1</sup>

The *CUX1* transcription factor regulates many genes including several involved in DNA replication and chromosome segregation.<sup>8</sup> Cell-based assays have established a role for *CUX1* in the control of cell-cycle progression, cell motility, and invasion.<sup>8</sup> Within the RNA-sequencing data obtained from the  $-7/\text{del}(7q)$  leukemia patients studied by McNerney et al there was enrichment of gene transcripts known to play a role in the mitotic cell cycle, many of which were direct targets of

*CUX1*.<sup>1</sup> Thus *CUX1* might act as a TSG in myeloid cells through the regulation of genes involved in the control of the cell cycle.

*CUX1* is highly conserved between humans and *Drosophila* and to investigate the hypothesis that *CUX1* acts as a TSG in vivo, McNerney et al performed animal modeling experiments using *Drosophila*. A targeted RNAi approach in developing *Drosophila* hemocytes demonstrated that haploinsufficiency of the ortholog *cut* resulted in increased hemocyte proliferation and melanotic tumor formation in developing larvae. Similarly, partial knockdown of *CUX1* in human hematopoietic progenitor cells resulted in increased hematopoietic engraftment upon transplantation into immunodeficient mice.<sup>1</sup> These data suggest that *cut/CUX1* acts as a TSG important in the regulation of normal hematopoietic cell growth in *Drosophila* and humans.

The study by McNerney et al provides good evidence that *CUX1* is a haploinsufficient TSG involved in malignant myeloid disorders with  $-7/\text{del}(7q)$ .<sup>1</sup> However, whether *CUX1* is a causal gene in relation to

producing a clonal human hematopoietic disorder, and whether haploinsufficiency of an additional gene (or genes) mapping to chromosome 7 play a role in disease pathogenesis, remain important questions. Deletions of chromosome 7q are typically large and it is highly likely that the loss of several genes along chromosome 7q will have various phenotypic effects in patients thus affected. Clearly, mouse knockout models might prove very informative in relation to this question. Mutation of other genes on 7q may also play an important role in disease pathogenesis. A good example is the *EZH2* gene, mapping to 7q36, mutation of which is associated with 7q uniparental disomy or 7q36.1 microdeletion, but not generally with monosomy 7 and  $\text{del}(7q)$ , in malignant myeloid disorders.<sup>9</sup> Whether *CUX1* is inactivated as a result of point mutations in malignant myeloid disorders remains to be fully determined; to date, only 1 missense mutation has been described in a secondary AML patient.<sup>10</sup>

Finally, in contrast to malignant myeloid disorders with abnormalities of chromosome 7, frequent over-expression of *CUX1* has been reported in solid tumors of various types, including breast tumors and cancer cell lines, and in this setting it has been proposed that elevated *CUX1* expression plays an important role in tumor progression.<sup>8</sup> Clearly, aberrant expression of *CUX1*, either increased or reduced compared with normal levels in a given cell type, may play an important role in human tumorigenesis: *CUX1* dosage matters!

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

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● ● ● PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS

Comment on Mildner et al, page 1016

## miR-142 keeps CD4<sup>+</sup> DCs in balance

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In this issue of *Blood*, Mildner et al report elucidation of a transcriptome-wide miRNA map for in vivo mononuclear phagocyte populations. Using this approach, they identify that the miRNA miR-142 was critical for dendritic cell (DC) development, particularly for homeostatic regulation of the CD4<sup>+</sup> DC subset.<sup>1</sup>

**D**endritic cells (DCs) are a rare type of white blood cell that play a critical role in bridging the innate and adaptive immune system. They are essential for presentation of both endogenous (self-) antigens and foreign (pathogen-derived) antigens and as such constitute a frontline defense against microorganisms. To effectively provide immune protection, different DC subsets have evolved.

The genetic program that coordinates the development of these subsets is controlled largely by cytokines and transcription factors.<sup>2</sup> Interferon regulatory factors (Irf), namely Irf4 and Irf8, are essential for the development of CD4<sup>+</sup> and CD8<sup>+</sup> DC lineages, respectively, in mice, and PU.1 is a pioneering transcription factor for myeloid cell development (see figure). While the fundamental differentiation

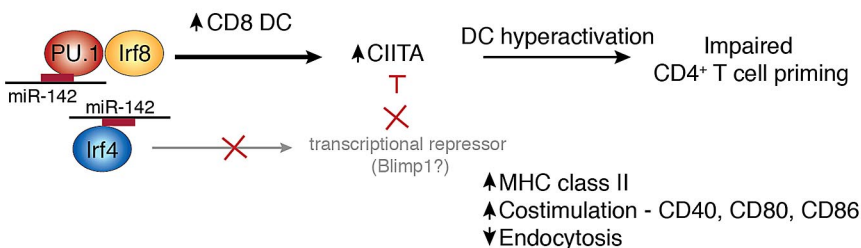
program for DCs is laid down by specific transcription factors, increasingly it is recognized that fine-tuning of this program is likely to depend on an added layer of control mediated through microRNA (miRNA) expression.<sup>3,4</sup>

MicroRNAs are a class of small noncoding RNAs that repress translation of target genes through their complementary binding to the 3' untranslated regions of mRNAs by repressing translation initiation, induction of transcript decapping and deadenylation, and degradation of the mRNA. Canonical miRNAs are initially transcribed as long primary transcripts that are then processed into mature 20-24 nucleotide (nt)-long miRNAs. In mammals, posttranscriptional modulation of gene expression depends on an imperfect matching between the miRNA and target. However, perfect complementarity between the 5'-proximal seed sequence of the miRNA with the mRNA target is thought to be particularly important in metazoans. This feature introduces a level of promiscuity in which each miRNA can bind multiple targets (hundreds to thousands) and reciprocally, each mRNA can be targeted by many different miRNAs. Some understanding of miRNA expression in DCs has been gained through investigation mainly of in vitro GM-CSF-derived DCs. This has resulted in identification of several miRNAs, mainly in human cultured DCs, that target cytokine production (miR-10a, miR-21, miR-142-3p), DC activation (miR-155 and miR-146), and antigen presentation (miR-511, miR-99b, miR-211). Understanding miRNA expression and requirements in different in vivo DC subsets, and which components of the differentiation and maturation pathways they target, has been a significant challenge. Deletion of the gene encoding Dicer, a key enzyme necessary for the biogenesis of miRNAs, specifically in CD11c<sup>+</sup> cells, resulted in only a minimal impact on DC differentiation.<sup>4</sup> These mice displayed a perturbed Langerhans cell compartment, but conventional DCs were relatively normal. The lack of phenotype observed in the conventional DC compartment may have been due to the fact that miRNAs are very stable. Combined with the quite short half-life of DCs, this is likely to result in insufficient time for the miRNAs to be efficiently depleted in mature cells. Thus, to fully evaluate the involvement of miRNAs in DC approaches to deplete miRNAs from earlier stages of differentiation will be important.

Before binding to miR-142



After PU.1/Irf4 binding



Potential model of the interactions between miR-142 and key transcription factors implicated in the pathway. At steady-state (top panel), the interplay between PU.1, Irf8, and Irf4 orchestrates the differentiation of CD4 and CD8 DCs. Heterodimeric binding of PU.1 and Irf8 induces expression of the MHC class II transactivator CIITA, which may be repressed by transcription factors such as Blimp1 to allow efficient induction of DC antigen presentation required for T-cell priming. Modulation of the PU.1/Irf4 complex by miR-142 (bottom panel) disrupts the generation of CD4 DCs and favors the generation of CD8 DCs. Because Irf4 is important for induction of other transcription factors including Blimp1, failure of CIITA repression leads to elevated DC activation and maturation and impaired CD4<sup>+</sup> T-cell priming ability. miR-142 may thus regulate the balance of different transcription factors to guide the outcome of an immune response.