and newer technology may be integrated in the future. Finally, we await a more detailed understanding of the biology of hyperdiploid ALL in the quest to develop targeted therapy.

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

REFERENCES

1. Paulsson K, Johansson B. High hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer.* 2009;48(8):637-660.

2. Dastugue N, Suciu S, Plat G, et al. Hyperdiploidy with 58-66 chromosomes in childhood B-acute lymphoblastic leukemia is highly curable: 58951 CLG-EORTC results. *Blood.* 2013;121(13):2415-2423.

3. Gordon DJ, Resio B, Pellman D. Causes and consequences of aneuploidy in cancer. *Nat Rev Genet.* 2012;13(3):189-203.

4. Paulsson K, Mörse H, Fioretos T, et al. Evidence for a single-step mechanism in the origin of hyperdiploid childhood acute lymphoblastic leukemia. *Genes Chromosomes Cancer.* 2005;44(2):113-122.

5. Heerema NA, Raimondi SC, Anderson JR, et al. Specific extra chromosomes occur in a modal number

dependent pattern in pediatric acute lymphoblastic leukemia. *Genes Chromosomes Cancer.* 2007;46(7):684-693.

6. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). *Blood.* 2007; 109(3):926-935.

7. Moorman AV, Ensor HM, Richards SM, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. *Lancet Oncol.* 2010; 11(5):429-438.

8. Sutcliffe MJ, Shuster JJ, Sather HN, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. *Leukemia.* 2005;19(5):734-740.

9. Ge Y, Haska CL, LaFiura K, et al. Prognostic role of the reduced folate carrier, the major membrane transporter for methotrexate, in childhood acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Clin Cancer Res.* 2007;13(2 pt 1):451-457.

10. Senovilla L, Vitale I, Martins I, et al. An immunosurveillance mechanism controls cancer cell ploidy. *Science.* 2012;337(6102):1678-1684.

● ● ● **HEMATOPOIESIS & STEM CELLS**

Comment on Malinge et al, page 2440

Ikaros, Notch, and GATA1 cross paths during megakaryopoiesis

Ivan Maillard[1]   [1]UNIVERSITY OF MICHIGAN

In this issue of *Blood*, Malinge et al[1] describe new reciprocal interactions between Ikaros, Notch, and GATA transcription factors during megakaryocyte development. Ikaros represses megakaryocytic genes and selected Notch targets, while being turned off by GATA1 upon terminal differentiation.

Both Ikaros and Notch proteins were first identified for their essential functions in lymphocyte development as well as for their dysregulation in lymphoid malignancies. The Krüppel-type zinc finger transcription factor Ikaros (encoded by *IKZF1*) regulates expression of multiple genes critical for the development of lymphoid lineages. In mice, Ikaros loss-of-function is associated with the emergence of aggressive T-cell lymphoblastic leukemias. In humans, high-risk B-cell acute lymphoblastic leukemias harbor recurrent genomic deletions at the *IKZF1* locus.[2] In addition, emerging work suggests that Ikaros also functions in the myeloid, erythroid, and megakaryocytic lineages. For example, an *Ikzf1-gfp* reporter allele revealed a strong

correlation between high Ikaros expression in hematopoietic progenitors and myeloid cell fate, while low Ikaros expression identified progenitors committed to megakaryocytic and erythroid differentiation.[3] Moreover, Ikaros-deficient mice have thrombocytosis, suggesting that Ikaros can negatively regulate megakaryopoiesis.

Notch signaling was first studied for its essential role in T-cell development. In addition, activating *NOTCH1* mutations are present in a high proportion of T-cell acute lymphoblastic leukemias in mice and humans.[4] In this context, Notch and Ikaros appear to have antagonistic functions. Expression of dominant-negative Ikaros isoforms lacking DNA binding can cooperate

with Notch activation in T-cell leukemia.[5] Ikaros may directly antagonize the effects of the Notch transcriptional activation complex at target gene loci. At least in mice, Ikaros can also repress transcription from internal *Notch1* promoter elements that drive expression of truncated constitutively active Notch receptors.[6,7] Interestingly, recent work indicates that Notch signaling also plays important functions in the myeloid and megakaryocytic lineages.[8,9] These observations have set the stage to investigate whether Notch and Ikaros interact and exert new functions outside of lymphoid progenitors.

In this issue, Malinge et al[1] focus their attention on the role of Ikaros in megakaryopoiesis. As a rationale to initiate this work, the authors built on their past observations that Notch signaling supports enhanced megakaryopoiesis in vitro and in vivo.[9] In addition, acute megakaryocytic leukemias (AMKL) carrying the recurrent OTT-MAL translocation were shown to activate an aberrant Notch signature.[10] Based on the hypothesis that Ikaros may antagonize Notch in these cells by analogy to lymphoid cells, Malinge et al[1] report that Ikaros overexpression inhibits both Notch-driven megakaryocyte specification and expansion of an AMKL cell line expressing the OTT-MAL fusion protein. When analyzing the mechanisms of this effect, however, the authors discovered that Ikaros had a broad impact on the megakaryocyte transcriptional network, including many Notch-independent effects. Together with other regulators of megakaryocytic differentiation, GATA1 expression was downregulated by Ikaros. Reciprocally, the abundance of Ikaros transcripts was reduced by GATA1 expression, while direct binding of GATA1 to the *Ikzf1* locus was identified by chromatin immunoprecipitation. These findings suggest that Ikaros expression is downregulated by the GATA2/GATA1 switch that occurs during megakaryopoiesis, perhaps to allow full expression of multiple genes repressed by Ikaros that are necessary for terminal differentiation. Interestingly, this phenomenon was specific to the megakaryocyte lineage and not observed in erythroid progenitors, in which Ikaros appeared to cooperate with GATA1 to promote erythroid differentiation. These observations suggest the existence of
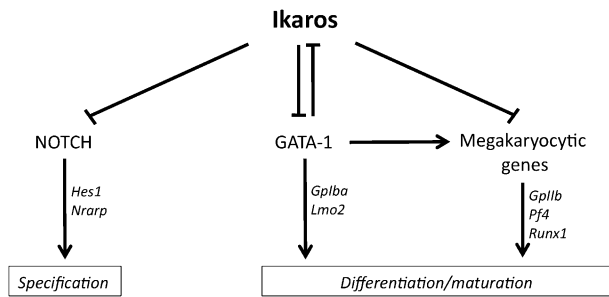
Regulation of megakaryopoiesis by Ikaros, Notch, and GATA-1. Findings from Malinge et al[1] indicate that Ikaros controls megakaryocyte development both by inhibiting Notch signaling and by repressing multiple megakaryocyte differentiation genes, including GATA-1. Conversely, GATA-1 downregulates Ikaros expression. See Figure 7 in the article by Malinge et al that begins on page 2440.

multiple lineage-specific effects of Ikaros. In support of this idea, Malinge et al[1] described a panel of putative direct Ikaros target genes in megakaryocyte progenitors that showed only limited overlap with genes previously identified as putative targets in thymocytes.

Altogether, findings reported in this paper expand the scope of Ikaros functions and unravel a critical transcriptional network at the heart of megakaryocytic differentiation (see figure). More work is needed to fully dissect the reciprocal interactions between nodes of this network, especially because some of these interactions could be indirect. Another remarkable finding is the high degree of lineage specificity with which Ikaros operates and regulates target genes. It is likely that differential usage of transcriptional cofactors and epigenetic regulators accounts for target gene specificity in individual lineages. Finally, although mostly focused on normal hematopoiesis, work presented by Malinge and colleagues could have implications for the understanding of malignant disease, in particular AMKL. Ikaros overexpression profoundly inhibited the expansion of an AMKL cell line. In contrast, Ikaros loss cooperated with Gata1 deficiency in mice to block erythroid development and increase the proliferation of megakaryocyte progenitors.[1] This constellation of findings could have oncogenic effects, for example, in AMKL carrying an enhanced Notch signature or in AMKL associated with Down syndrome, in which loss-of-function *GATA1* mutations are typically observed. Thus, it could be interesting to study the pattern of Ikaros isoforms expressed in AMKL (as dominant-negative isoforms could block Ikaros activity) and the status of the *IKZF1*

locus in AMKL subtypes (because genomic deletions could be present, as previously reported in B-cell acute lymphoblastic leukemia and more recently in myeloid malignancies). In hematopoiesis, it appears that the wings of Ikaros have not fallen off, and we should continue the journey toward new discoveries.

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

REFERENCES

1. Malinge S, Thiollier C, Chlon TM, et al. Ikaros inhibits megakaryopoiesis through functional interaction with GATA-1 and NOTCH signaling. *Blood.* 2013;121(13):2440-2451.

2. Mullighan CG, Su X, Zhang J, et al; Children's Oncology Group. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *N Engl J Med.* 2009;360(5):470-480.

3. Yoshida T, Ng SY, Zuniga-Pflucker JC, et al. Early hematopoietic lineage restrictions directed by Ikaros. *Nat Immunol.* 2006;7(4):382-391.

4. Van Vlierberghe P, Ferrando A. The molecular basis of T cell acute lymphoblastic leukemia. *J Clin Invest.* 2012; 122(10):3398-3406.

5. Beverly LJ, Capobianco AJ. Perturbation of Ikaros isoform selection by MLV integration is a cooperative event in Notch(IC)-induced T cell leukemogenesis. *Cancer Cell.* 2003;3(6):551-564.

6. Jeannet R, Mastio J, Macias-Garcia A, et al. Oncogenic activation of the Notch1 gene by deletion of its promoter in Ikaros-deficient T-ALL. *Blood.* 2010; 116(25):5443-5454.

7. Gómez-del Arco P, Kashiwagi M, Jackson AF, et al. Alternative promoter usage at the Notch1 locus supports ligand-independent signaling in T cell development and leukemogenesis. *Immunity.* 2010; 33(5):685-698.

8. Klinakis A, Lobry C, Abdel-Wahab O, et al. A novel tumour-suppressor function for the Notch pathway in myeloid leukaemia. *Nature.* 2011;473(7346):230-233.

9. Mercher T, Cornejo MG, Sears C, et al. Notch signaling specifies megakaryocyte development from hematopoietic stem cells. *Cell Stem Cell.* 2008;3(3): 314-326.

10. Mercher T, Raffel GD, Moore SA, et al. The OTT-MAL fusion oncogene activates RBPJ-mediated transcription and induces acute megakaryoblastic leukemia in a knockin mouse model. *J Clin Invest.* 2009;119(4): 852-864.

● ● ● **PHAGOCYTES, GRANULOCYTES, & MYELOPOIESIS**

CARD9: at the intersection of mucosal and systemic antifungal immunity

Michail S. Lionakis[1] and Steven M. Holland[1] [1]NATIONAL INSTITUTES OF HEALTH

In this issue of *Blood*, Drewniak et al[1] demonstrate that neutrophil killing of *Candida* depends on caspase recruitment domain–containing protein 9 (CARD9) function but not on the production of reactive oxidants. Furthermore, although CARD9 is required for *Candida* killing, it is dispensable for killing of *Staphylococcus aureus* and *Aspergillus*.

Candida inhabits the mucosal surfaces in ~50% of healthy individuals, typically without sign or symptom. However, when weakening or perturbation of host defenses occurs, it can cause a range of ailments, from localized mucosal disease to fatal systemic infections. Until now, the host factors involved in mucosal and systemic disease were not overlapping. Adaptive immunity, predominantly in the form of interleukin-17

(IL–17)–producing lymphocytes, controls *Candida* at the mucosal level by promoting the production of neutrophil-recruiting chemotactic factors and the generation of epithelial anti-*Candida* antimicrobial peptides.[2] In stark contrast, innate immunity controls systemic candidiasis through oxidative and nonoxidative phagocyte pathways. Consistent with this dichotomy, patients with AIDS develop mucosal but not