blood

2013 122: 3391-3392
doi:10.1182/blood-2013-09-527606

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

inside blood®
14 NOVEMBER 2013 | VOLUME 122, NUMBER 20

HEMATOPOIESIS & STEM CELLS

Comment on Takai et al, page 3450

A new twist to the GATA switch

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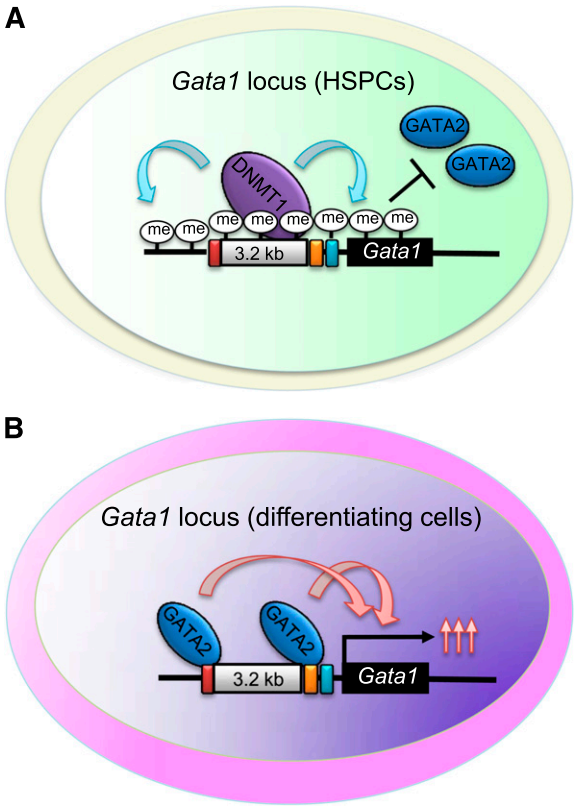
In this issue of *Blood*, Takai et al provide some tantalizing clues on how expression of the GATA1 transcription factor, a master regulator of erythroid/megakaryocytic differentiation, is suppressed in the hematopoietic stem/progenitor cell (HSPC) compartment.[1]

Suppression is important because forced expression of GATA1 in the stem cell compartment leads to loss of self-renewal capacity.[2] Members of the GATA factor family of transcription factors have distinct and essential roles in hematopoiesis.[3] GATA2 is essential for maintenance of the HSPC compartment, but it is also involved in the initial activation of GATA1 expression at the first steps of erythroid/megakaryocytic differentiation. This is referred to as the GATA factor switch.[4] A conceptual problem of this mechanism is that GATA1 expression is suppressed in HSPCs, which are dependent on GATA2 activity. So why is GATA2 unable to activate GATA1 expression in HSPCs? Previous work has shown that the dynamic spatiotemporal regulation of GATA factor levels is more important than their identity. When expressed under the control of the appropriate regulatory elements of the *Gata1* locus, GATA2 and GATA3 transgenes rescue the lethal erythroid defects of GATA1 *null* mice.[2] Thus, the developmental control mechanism of GATA1 expression is hard-wired in *cis*-regulatory elements residing in the *Gata1* locus.



Model for the initiation of differentiation through stage-specific *Gata1* gene regulation. (A) In HSPCs, DNMT1 is recruited to the 3.2-kb sequence intervening the *Gata1* gene enhancer sequences (red, G1HE; orange, double GATA sites; and blue, CACC box) and confers *Gata1* gene inactivation by maintaining DNA methylation. GATA2 is unable to bind to the inactivated *Gata1* gene. (B) Upon initiation of differentiation, DNA methylation decreases and GATA2 transactivates *Gata1* gene expression. me, methylation.

These *cis*-regulatory elements have been mapped in detail through analysis of reporter transgenes and also, to a more limited extent, through introduction of mutations in the endogenous *Gata1* locus. A 3.7-kb fragment in front of the hematopoietic cell–specific *Gata1* promoter contains a number of such elements. The *Gata1* gene hematopoietic enhancer (G1HE) is located at the promoter-distal part of this fragment, while double GATA sites and a CACC motif are found in the promoter-proximal part. All 3 elements are indispensable for erythroid cell–specific gene expression. The intervening 3.2-kb DNA fragment is of as-yet undetermined significance. Indeed, Takai et al show that combining the 3 positive regulatory elements in a 659-bp fragment is sufficient to recapitulate hematopoietic cell–specific GATA1 expression in transgenic mice. It would therefore appear that the intervening 3.2-kb DNA fragment is dispensable for appropriate regulation of GATA1 expression. However, the authors go further and discover

that there is an unexpected twist to this story. They make full use of the green fluorescent protein (GFP) that they have inserted into the *Gata1* gene to serve as a reporter for gene activation. As expected, they observe that, in the context of a wild-type *Gata1* reporter transgene, expression of GFP is suppressed in the HSPC compartment.

In contrast, *Gata1* reporter transgenes lacking the intervening 3.2-kb DNA fragment but retaining the 3 positive regulatory elements express GFP abundantly in this compartment. This striking difference in GFP expression is most graphically illustrated in Figure 3, panels D and F, of their article (compare wild-type transgene, G1B-GFP, with the mutant transgene, MG-GFP). This result demonstrates that the intervening 3.2-kb DNA fragment is required for suppression of the Gata1 gene in the HSPC compartment. But how is this achieved? A survey of epigenetic modifications has revealed that the *Gata1* locus is heavily methylated in the HSPC compartment.[5] DNA methylation, occurring at cytosine residues in the dinucleotide sequence 5′-CG-3′, is a very stable modification which is strongly associated with repression of gene activity. Takai et al analyze DNA methylation of the transgenes in HSPCs. In the case of the wild-type transgene, they find high methylation levels at the *Gata1* promoter and regulatory elements. In contrast, DNA methylation was much reduced in the mutant transgene lacking the intervening 3.2-kb DNA fragment. This fragment is therefore required for high DNA methylation levels of critical *Gata1* regulatory elements in HSPCs. Hypermethylated DNA attracts repressor proteins including DNA methyltransferase 1 (DNMT1). DNMT1 ensures that DNA methylation patterns are faithfully passed on to the daughter cells after cell division, thus locking the *Gata1* gene stably in an inactive configuration. The authors provide evidence that under these conditions GATA2 is unable to bind to the *Gata1* regulatory elements, providing a mechanistic explanation for the inability of GATA2 to activate the *Gata1* gene in HSPCs (see figure). To proceed to lineage commitment and differentiation would require demethylation of the *Gata1* locus, and it remains to be investigated how this is achieved. This could be through a passive process, involving failure to maintain methylation after DNA replication.

Interestingly, Takai et al characterize a binding site for E2F transcription factors which provides a potential lead. In quiescent cells, E2F factors form a repressive complex including the Retinoblastoma protein and DNMT1.[6] When the cells enter the cell cycle, the Retinoblastoma protein is phosphorylated and the repressive complex is released, allowing E2F to become active. If the repressive complex is not reformed after cell division, DNMT1 would fail to maintain DNA methylation and the methylation marks would be lost. Alternatively, demethylation might be an active process, for which a number of different mechanisms have been proposed[7] including oxidation of the methyl groups by TET proteins, modification by AID/APOBEC enzymes, and base excision DNA repair. Aberrant DNA methylation is a hallmark of many cancers including hematologic malignancies, and DNA methylation inhibitors are used to treat patients with myelodysplastic syndromes. The results described by Takai et al suggest that restoration of expression of genes required for differentiation, such as *GATA1*, contributes to the therapeutic efficacy of DNA methylation inhibitors.

Finally, the current study relies on the use of transgenes, which are analyzed in the presence of the endogenous *Gata1* locus. An important next step would be to assess the role of the elements identified in the context of the endogenous locus. Although this used to be a mammoth task, the flurry of recent articles describing CRISPR/CAS9 as a very efficient tool for mammalian genome engineering[8] makes this a realistic proposition. Such experiments will provide an increasingly detailed picture of the dynamic regulation of endogenous GATA factor expression during hematopoiesis.

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

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Comment on Casucci et al, page 3461

Risky business: target choice in adoptive cell therapy

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In this issue of *Blood*, Casucci et al present an elegant study that describes a potential new target for adoptive cell transfer (ACT), in this case CD44 splice variant 6 (CD44v6), and detail why it may be a good target for ACT and how to manage expected off-tumor/on-target toxicities.[1]

There has been an explosion of interest in field of adoptive cell transfer due to the recent clinical success reported by several groups in the treatment of B cell malignancies.[2-4] Durable clinical responses have been documented in several patients, including those heavily pretreated with standard chemotherapy, using T cells genetically