Regulation of LMO2 mRNA and protein expression in erythroid differentiation

Stephen J. Brandt[1,2] and Mark J. Koury[1,2]

[1]Department of Medicine, Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN, USA; [2]Medical Service, VA Tennessee Valley Healthcare System, South Nashville, TN, USA. E-mail: stephen.brandt@vanderbilt.edu and mark.koury@vanderbilt.edu. *doi:10.3324/haematol.2008.005140*

Erythropoiesis, the process by which mature red blood cells differentiate from hematopoietic stem cells, provides about 2×10^{11} new erythrocytes daily to replace the 1% of old cells removed from the circulation. Red cell production increases several-fold after blood loss or hemolysis but does not overshoot because it is so tightly regulated. This regulation involves three basic processes in erythroid progenitor and precursor cell populations: proliferation, differentiation, and survival. Although they act in concert from the earliest cell committed to erythropoiesis, the burst-forming unit-erythroid (BFU-E), through the last cell division of erythroblasts, each of these three processes can be regulated independently of another. After blood loss or hemolysis, stem cell factor (SCF/Kit-ligand) and glucocorticoids increase proliferation of cells in the BFU-E to colony-forming unit-erythroid (CFU-E) stages.[1] However, SCF and glucocorticoids have little effect on survival or differentiation of these erythroid progenitor cells. Erythopoietin (EPO), the major physiologic regulator of erythropoiesis, is regulated by hypoxia at the level of its transcription.[2] EPO promotes the survival of erythroid cells in the CFU-E through basophilic erythroblast stages without affecting their proliferation or differentiation.

Contact with macrophages in erythroblastic islands regulates proliferation of these EPO-dependent cells without affecting their survival or differentiation.[3] Differentiation of late-stage erythroblasts is characterized by the synthesis and accumulation of hemoglobin, the most abundant and major functional protein of mature erythrocytes. Accumulation of heme and globin chains, which is finely regulated to assure erythroblast survival,[4,5] has no effect on cellular proliferation, which has largely ceased by this stage of differentiation.

Proliferation, differentiation, and survival of erythroid progenitors are ultimately controlled through activation and repression of specific genetic programs. An important transcriptional regulator in erythropoiesis is LIM domain-only protein 2 (LMO2). Originally discovered from its involvement by chromosomal translocation in T-cell acute lymphoblastic leukemia, the *LMO2* gene encodes a small nuclear protein with little beyond its two LIM domains. This LIM domain, while related structurally to the zinc finger DNA-binding domains of the GATA family of transcription factors, is involved in protein-protein rather than protein-DNA interactions. LMO2 and related LIM-only proteins appear to function as adaptor molecules and assist in the assembly of multimeric transcription factor complexes.

The first indication that LMO2 was important for erythroid differentiation came from gene targeting studies in embryonic stem (ES) cells. Homozygous inactivation of the *Lmo2* gene in mice led to the complete absence of yolk sac erythropoiesis and early embryonic lethality.[6] Further, both *Lmo2*$^{-/-}$ embryonic stem (ES) cells[6] and wild-type ES cells transduced with an anti-LMO2 single-chain antibody[7] were completely blocked in erythroid differentiation. In contrast, enforced Lmo2 expression was reported to inhibit the differentiation of erythroid progenitor cell lines.[8] However, while LMO2 protein could have opposite actions in early- *versus* late-stage erythropoiesis, it more likely has dominant negative effects when overexpressed.[9]

Given its significance in erythropoiesis, the mechanism by which LMO2 is regulated in the cell is an important subject. It is noteworthy, therefore, that Felli *et al.*[10] report in a paper in this issue of the journal that microRNA 223 (miR-223) reduces expression of LMO2 message and protein. miRNAs are small non-coding RNAs that affect gene expression at a post-transcriptional level by both promoting the degradation of specific mRNAs and inhibiting their translation. The authors found, first, that miR-223 expression declined during erythroid differentiation of cord blood CD34^{+} cells, while *LMO2* was upregulated. They then showed that miR-223 interacted with the 3' untranslated region of *LMO2* mRNA and that forced expression of miR-223 reduced LMO2 message and protein and inhibited erythroid differentiation. The paper suggests that down-modulation of miR-223 is required for erythroid differentiation and establishes LMO2 as one of its major targets (Figure 1A). Two other studies in press also found that miRNA-223 down-regulates LMO2 expression.[11,12]

LIM-only and LIM-homeodomain (LIM-HD) proteins interact with the LIM domain-binding protein Ldb1, and it is clear from studies of their *Drosophila* orthologs that the proper stoichiometry of Ldb1 and its interaction partners is essential for their biological actions. Ldb1 in turn interacts with two single-stranded DNA-binding proteins (SSBPs), SSBP2 and SSBP3, which together contribute to a common DNA-binding complex. This complex, which also contains the transcription factors GATA-1, Tal1, and one of Tal1's E protein DNA-binding partners, binds a bipartite E box-GATA sequence motif in the regulatory regions of its target genes.[13] When overexpressed in a murine erythroid cell line, the SSBPs increased endogenous Ldb1 and Lmo2 levels, the DNA-binding activity of this multiprotein complex, and expression of select target genes.[14] SSBP2 was shown to inhibit Ldb1 and Lmo2 interaction with an E3 ubiquitin ligase, RING Finger LIM Domain-Binding Protein (RLIM), preventing RLIM-mediated ubiquitination of Ldb1 and protecting Ldb1 and Lmo2 from proteasomal degradation.[14] Importantly, Ldb1 and Lmo2 protein abundance changed in the opposite direction with SSBP overexpression and knockdown, suggesting that the SSBPs

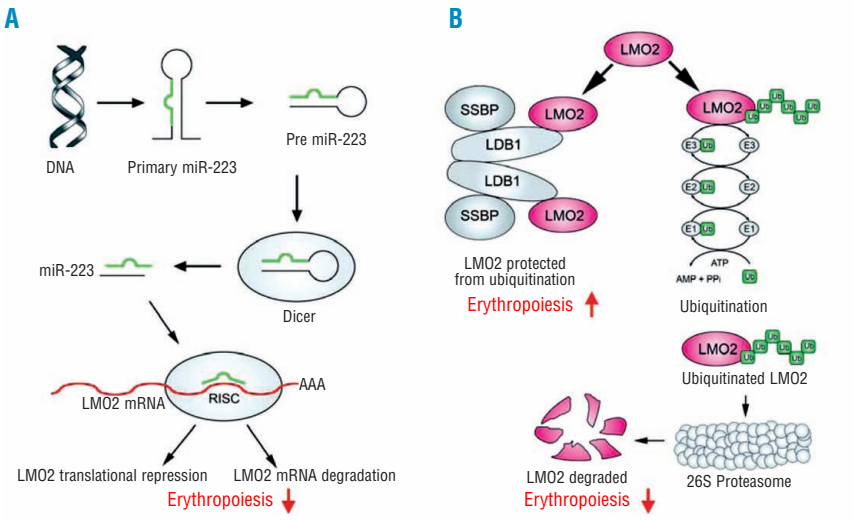

Figure 1. Regulation of LMO2 in erythropoiesis. (A) Regulation of *LMO2* mRNA by miRNA-223. Specific binding of this small non-coding RNA to the *LMO2* 3' untranslated region inhibits the translation of this mRNA and promotes its degradation, with consequences for erythroid differentiation. (B) Regulation of LMO2 protein by single-stranded DNA-binding proteins (SSBPs). These SSBPs inhibit LMO2 and LIM Domain-Binding Protein 1 (Ldb1) interaction with an E3 ubiquitin ligase, RING Finger LIM Domain-Binding Protein (RLIM), sparing them from proteasomal destruction and increasing their steady state concentrations in the cell RISC, RNA-induced silencing complex.

set the levels of Ldb1 and Lmo2 during erythropoiesis (Figure 1B).

Finally, recent studies have documented recruitment of the E box-GATA-binding complex to the beta-globin locus control region and proximal promoter, with additional data suggesting it regulates globin gene transcription through an effect on DNA looping.[15] Of considerable interest given its own regulation by an miRNA, LMO2 was recently implicated in down-regulating miRNA-142.[16]

In summary, recent studies, including the one reported by Felli *et al.*[10], have shown that LMO2 is regulated at both a posttranscriptional and post-translational level in erythroid progenitors. This undoubtedly contributes to the precision with which erythrocyte production is controlled *in vivo*.

Drs. Stephen Brandt and Mark Koury are Professors of Medicine at Vanderbilt University.

Work described in this manuscript was supported by Merit Review Awards from the Department of Veterans Affairs (SJB and MJK) and by National Institutes of Health grant R01 HL49118 (SJB).

References

1. von Lindern M, Schmidt U, Beug H. Control of erythropoiesis by erythropoietin and stem cell factor: A novel role for Bruton's tyrosine kinase. Cell Cycle 2004;3:876-9.
2. Koury MJ. Erythropoietin: The story of hypoxia and a finely regulated hematopoietic hormone. Exp Hematol 2005; 33:1263-70.
3. Rhodes MM, Kopsombut P, Bondurant MC, Price JO, Koury MJ. Adherence to macrophages in erythroblastic islands enhances erythroblast proliferation and increases erythrocyte production by a different mechanism than erythropoietin. Blood 2008;111:1700-8.
4. Chen JJ. Regulation of protein synthesis by the heme-regulated eIF2alpha kinase: Relevance to anemias. Blood 2007; 109:2693-9.
5. Weiss MJ, Zhou S, Feng L, Gell DA, Mackay JP, Shi Y, et al. Role of alpha-hemoglobin-stabilizing protein in normal erythropoiesis and beta-thalassemia. Ann NY Acad Sci 2005; 1054:103-17.
6. Warren AJ, Colledge WH, Carlton MB, Evans MJ, Smith AJ, Rabbitts TH. The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. Cell 1994;78:45-57.
7. Nam CH, Lobato MN, Appert A, Drynan LF, Tanaka T, Rabbitts TH. An antibody inhibitor of the LMO2-protein complex blocks its normal and tumorigenic functions. Oncogene 2008;27:4962-8.
8. Visvader JE, Mao X, Fujiwara Y, Hahm K, Orkin SH. The LIM-domain binding protein Ldb1 and its partner LMO2 act as negative regulators of erythroid differentiation. Proc Natl Acad Sci USA 1997;94:13707-12.
9. Terano T, Zhong Y, Toyokuni S, Hiai H, Yamada Y. Transcriptional control of fetal liver hematopoiesis: Dominant negative effect of the overexpression of the LIM domain mutants of LMO2. Exp Hematol 2005;33:641-51.
10. Felli N, Pedini F, Romania P, Biffoni M, Morsilli O, Castelli G, et al. MicroRNA 223 dependent expression of LMO2 regulates normal erythropoiesis. Haematologica 2009;94: 479-486.
11. Malumbres R, Sarosiek KA, Cubedo E, Ruiz JW, Jiang X, Gascoyne RD, et al. Differentiation-stage-specific expression of microRNAs in B-lymphocytes and diffuse large B-cell lymphomas. Blood 2008. Epub ahead of print as doi:10.1182/blood2008-10-184077.
12. Yuan JY, Wang F, Yu J, Yang GH, Liu XL, Zhang JW. MicroRNA-223 reversibly regulates erythroid and megakaryocytic differentiation of K562 cells. J Cell Mol Med 2008. Epub as doi:10.1111/j.1582-4934.2008.00585.
13. Wadman IA, Osada H, Grütz GG, Agulnick AD, Westphal H, Forster A, et al. The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. EMBO J 1997;16:3145-57.
14. Xu Z, Meng X, Cai Y, Liang H, Nagarajan L, Brandt SJ. Single-stranded DNA-binding proteins regulate the abundance of LIM domain and LIM domain-binding proteins. Genes Dev 2007;21:942-55.
15. Song SH, Hou C, Dean A. A positive role for NLI/Ldb1 in long-range beta-globin locus control region function. Mol Cell 2007;28:810-22.
16. Yuan W, Sun W, Yang S, Du J, Zhai CL, Wang ZQ, et al. Downregulation of microRNA-142 by proto-oncogene LMO2 and its co-factors. Leukemia 2008;22:1067-71.