The ins and outs of erythroid heme transport

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Heme plays critical roles in erythropoiesis not only as a structural component of hemoglobin but also as a regulator of erythroid proliferation by affecting the expression of proteins involved in iron transport and globin synthesis.[1] Within the cell, heme is synthesized in the mitochondria and is subsequently transported to the cytosol for incorporation into hemoglobin and other hemoproteins. Because excess free heme is cytotoxic, intracellular heme must be maintained at levels that support function while preventing cellular damage. There is growing evidence that the tight control of intracellular heme levels is accomplished by coordination of heme synthesis, degradation, and trafficking between intracellular compartments.[2] In contrast to the well-characterized biochemistry of heme synthesis and degradation, the mechanisms of heme transport remain somewhat obscure.

Feline leukemia virus subgroup C receptor (*Flvcr1*) is the cell-surface receptor for a retrovirus that causes pure red cell aplasia in cats. It was first cloned by Janis Abkowitz's group, who later demonstrated that *Flvcr1* exports heme and is essential for fetal erythropoiesis using *Flvcr1*-null mice.[3,4] Recently, Emanuela Tolosano and colleagues showed that human *Flvcr1* encodes two distinct transcripts: a cell surface isoform *Flvcr1a* and a shorter, mitochondrial isoform that lacks the first exon, termed *Flvcr1b*.[5] This group previously generated mice with a germline deletion of *Flvcr1a* that retains *Flvcr1b* expression and found normal erythroid development in *Flvcr1a*-null embryos.[5] This is in sharp contrast to the lethal erythroid differentiation block seen in mice lacking both *Flvcr1* isoforms,[4] suggesting that the *Flvcr1b* is able to support erythroid differentiation in the absence of *Flvcr1a* and the erythroid defect in *Flvcr1a/1b*-null mice could be a result of cytosolic heme deficiency caused by the loss of *Flvcr1b*, rather than heme accumulation due to *Flvcr1a* deletion. While the mouse *Flvcr1* knockout models strongly suggest a dispensable role of *Flvcr1a* in fetal erythropoiesis, it cannot be excluded that *Flvcr1a* may be important in other stages of erythropoiesis. Indeed, the function of *Flvcr1b* inferred from the *Flvcr1a* mutant phenotype had not been verified by *in vivo* models of *Flvcr1b* deficiency.

In this issue of *Haematologica*, Mercurio and colleagues build on their previous analysis of germline *Flvcr1a* knockout mice[5] and continue to dissect roles of *Flvcr1a* and *Flvcr1b* isoforms in erythroid tissue by using mouse, zebrafish and cell culture models.[6] In mice and zebrafish, deletion of *Flvcr1a* resulted in anemia with a block in erythroid expansion, suggesting that *Flvcr1a* is required for the proliferation of erythroid progenitor cells. Although there are no data on *Flvcr1b*-specific knockouts, a distinct role of *Flvcr1b* in erythroid maturation can be deduced from the terminal maturation arrest in mouse bone marrow lacking both *Flvcr1a/1b* and the failure of *Flvcr1a* supplementation to restore the erythroid differentiation defect in *Flvcr1a/1b*-null zebrafish. In addition, heme depletion and supplementation in zebrafish morphants rescued erythroid defects associated with the loss of *Flvcr1a* and *Flvcr1a/1b*, providing indirect evidence for a causal relationship between cytosolic heme accumulation/deficiency and the erythroid phenotype. Knockdown experiments in human K562 cells further established a direct link between *Flvcr1a* and *Flvcr1b* silencing with cytosolic and mitochondrial heme accumulation and erythroid development defects. Taken together, these data suggest that *Flvcr1a* and *Flvcr1b* are involved in distinct stages of erythropoiesis and likely function as safety valves to maintain optimal cytosolic heme levels during erythroid development.

The discovery of cellular heme transporters such as *Flvcr1a* and *Flvcr1b* reveals the exquisite mechanisms in controlling heme balance. However, many questions remain regarding the function and cellular characteristics of *Flvcr1* isoforms in erythroid tissue. First, the critical role of *Flvcr1b* in erythroid maturation is largely inferred from animal models of *Flvcr1a* deficiency. This speculation needs to be verified in animals with specific deletion of *Flvcr1b* with intact *Flvcr1a* expression. Second, information on the subcellular localization of *Flvcr1* isoforms was obtained from non-erythroid cells overexpressing the proteins. It is unclear whether erythroid cells share this expression pattern. Tissue-specific *Flvcr1b* is suggested by differential *Flvcr1b* expression in erythroid cells and hepatocytes in response to ablation of the *Flvcr1a*-specific exon 1. Conditional deletion of exon 1 in mouse bone marrow reported in this paper results in loss of *Flvcr1a* but also of *Flvcr1b*, while liver-specific deletion with the same targeted allele has no impact on *Flvcr1b* expression.[7] Little is known about the regulation of *Flvcr1* and how intracellular heme transport coordinates with heme synthesis and degradation to achieve optimal intracellular heme balance throughout erythroid development. Finally, to fully understand the physiological significance of *Flvcr1* at the systemic level, the role of *Flvcr1* isoforms in non-erythroid tissues with high heme trafficking activity, such as duodenum and reticuloendothelial macrophages, as well as their contribution to local and systemic heme homeostasis are important issues to address in future studies.

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