Pharmacologic Induction of Fetal Hemoglobin Production

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• Fetal hemoglobin expression • Gene regulation • Globin
• Sickle cell • Thalassemia

Human hemoglobin is a tetrameric molecule comprised of two pairs of identical polypeptide subunits, each pair encoded by a different family of genes. The human α-like globin genes (ζ, α1, and α2) are located on chromosome 16 and the β-like globin genes (ε, $^{G}\gamma$, $^{A}\gamma$, δ, and β) are located on chromosome 11. During development, sequential switches take place in both the α- and β-like globin clusters that result in the production of six different types of hemoglobins. Synthesis of hemoglobin begins during the first weeks of embryonic development in the yolk sac where three different hemoglobins are made: (1) Hb Gower 1 ($\zeta_2\varepsilon_2$); (2) Hb Portland ($\zeta_2\gamma_2$); and (3) Hb Gower 2 ($\alpha_2\varepsilon_2$). By the 13th week of gestation, the site of erythropoiesis shifts from the embryonic yolk sac to the fetal liver where fetal hemoglobin (HbF; $\alpha_2\gamma_2$) starts to accumulate as the embryonic ε-globin gene is turned off. Shortly before birth, a second switch occurs as the site of erythropoiesis shifts from the fetal liver to the bone marrow. As a result of this second switch, the two adult hemoglobins (HbA, $\alpha_2\beta_2$; and HbA_2, $\alpha_2\delta_2$) gradually replace HbF. By the end of the first year of life, the hemoglobin composition is approximately 97.5% HbA, 2% HbA_2, and 0.5% HbF.

In adult life, the residual amounts of HbF are distributed unevenly among red cells. HbF is concentrated in a subpopulation of erythrocytes known as "F cells." The amount of HbF and the number of F cells are genetically determined and vary within a relatively narrow range in normal individuals. Such factors as acute blood loss, pregnancy, and bone marrow transplantation that lead to erythropoietic stress can result in an increase in baseline levels of HbF. In addition, inheritance of a variety of genetic

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determinants, such as sickle cell disease (SCD), thalassemia, and hereditary persistence of HbF, can also lead to higher levels of HbF and F cells in adult life.[1]

The levels of HbF in erythrocytes account for a large part of the clinical heterogeneity observed in patients with SCD[2,3] and β-thalassemia.[4,5] Patients with SCD from certain regions of Saudi Arabia[6] and India[7] have an unusually mild clinical sickling disorder associated with high levels of HbF. Moreover, the large multicenter study of the natural history of SCD (Cooperative Study of SCD) identified HbF as a major predictor of several complications, including painful events,[2,8] acute chest syndrome,[9] and mortality.[10] In addition, patients with SCD or β-thalassemia who co-inherit a genetic determinant for hereditary persistence of HbF usually have a mild clinical disorder.[4,5] These clinical and epidemiologic observations provided important clues about the beneficial role of HbF in modulating the pathophysiology of these disorders. In SCD, a high level of HbF interferes with the polymerization of HbS and prevents sickling of red blood cells. On the other hand, in β-thalassemia, a high level of γ-globin chain synthesis decreases non-α:α chain imbalance and ameliorates the anemia. Based on all these observations, it was proposed that pharmacologic induction of HbF production might be an effective therapeutic strategy for ameliorating the severity of SCD and β-thalassemia.

REGULATION OF γ-GLOBIN GENE EXPRESSION

Gene transcription of the γ-globin genes, and of other globin genes present in the β-globin cluster, is controlled by complex molecular mechanisms involving *cis*-acting elements, represented by specific nucleotide sequences, such as the cluster control region (LCR) and the promoters of the different globin genes and *trans*-acting elements, such as transcription factors and chromatin remodeling proteins.

The LCR consists of at least five DNase I hypersensitive sites (HS1–HS5). Each HS contains one or more binding motifs for three erythroid-specific transcription factors (GATA-1, NF-E2,[11–13] and EKLF[14,15]) in addition to binding sites for ubiquitous DNA binding proteins. HS1 to HS4 are formed only in erythroid cells.[16–18] The transcriptional-enhancer activity of the LCR resides mostly in HS2 and HS3. HS3 is believed to be involved in γ-globin activation during fetal-stage development[19] and in β-globin activation during adult life.[20] It is postulated that EKLF bound to HS3 may provide a competitive advantage for the interaction of the LCR with the β-globin promoter over its interactions with the γ-globin promoter, facilitating hemoglobin switching after birth.[21] The role of the LCR in switching has been conceptualized by the competition model based on the presence of developmental stage-specific transcription factors that mediate its interactions with the individual globin gene promoters.[22–24] However, significant uncertainty exists regarding the potential effects of the LCR on chromatin conformation in the β-globin gene cluster. In patients with Hispanic δβ-thalassemia in which the LCR is deleted, the β-globin cluster chromatin domain is in a closed, DNase I-resistant, transcriptionally inactive conformation, suggesting that the LCR functions to open chromatin in addition to its direct role in globin gene activation.[25] However, when the β-globin LCR was deleted from the endogenous mouse β-globin cluster in embryonic stem cells and somatic cell lines, β-like globin transcript levels were reduced, whereas the switching pattern during development remained normal and the chromatin of the β-globin gene cluster existed in an open DNase I–sensitive conformation.[26] These studies suggest that the LCR is not necessary for the establishment of an open chromatin cluster and that its primary function is that of an enhancer for transcriptional activation of the globin genes. Furthermore, these studies also suggested that the sequences conferring developmental stage-specific expression reside