Epigenetic regulation of fetal globin gene expression in adult erythroid cells

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The developmental regulation of globin gene expression has served as an important model for understanding higher eukaryotic transcriptional control mechanisms. During human erythroid development, there is a sequential switch from expression of the embryonic ε-globin gene to the fetal γ-globin gene in utero, and postpartum the γ-globin gene is silenced, as the β-globin gene becomes the predominantly expressed locus. Because the expression of normally silenced fetal γ-type globin genes and resultant production of fetal hemoglobin (HbF) in adult erythroid cells can ameliorate the pathophysiological consequences of both abnormal β-globin chains in sickle cell anemia and deficient β-globin chain production in β-thalassemia, understanding the complex mechanisms of this developmental switch has direct translational clinical relevance. Of particular interest for translational research are the factors that mediate silencing of the γ-globin gene in adult stage erythroid cells. In addition to the regulatory roles of transcription factors and their cognate DNA sequence motifs, there has been a growing appreciation of the role of epigenetic signals and their cognate factors in gene regulation, and in particular in gene silencing through chromatin. Much of the information about epigenetic silencing stems from studies of globin gene regulation. As discussed here, the term epigenetics refers to postsynthetic modifications of DNA and chromosomal histone proteins that affect gene expression and can be inherited through somatic cell replication. A full understanding of the molecular mechanisms of epigenetic silencing of HbF expression should facilitate the development of more effective treatment of β-globin chain hemoglobinopathies. (Translational Research 2015;165:115–125)

Abbreviations: Adox = adenosine-2′,3′-dialdehyde; BCL11A = B-cell lymphoma/leukemia A; CID = chemical inducer of dimerization; CoREST = REST-co-repressor; DNMT = DNA methyltransferase; EKLF = erythroid Krüppel-like factor; FoP = friend of PRMT1 (protein arginine methyltransferase); HbA = adult hemoglobin; HbF = fetal hemoglobin; HDAC = histone deacetylase; KLF1 = Krüppel-like factor 1; LCR = locus control region; LSD1 = lysine-specific demethylase 1; MBD = methylcytosine-binding domain; Mi2β/CHD4 = chromodomain helicase DNA–binding protein 4; NCoR/SMRT = nuclear receptor co-repressor-1/silencing mediator for retinoid and thyroid receptors; NuRDe = nucleosome remodeling and deacetylase; PCAF = P300-associated factor; PRMT = protein arginine methyltransferase; siRNA = small inhibitory RNA; shRNA = short hairpin RNA; TR2/TR4/DRED = nuclear receptor TR2/TR4 complex; β-YAC = beta-globin yeast artificial chromosome

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INTRODUCTION

DNA methylation was the first well-described epigenetic signal and was long posited to have a role in gene regulation.[1-3] Vertebrate globin genes were among the first in which an inverse relationship between cytosine methylation and transcription was demonstrated.[4-7] Both histone and nonhistone chromosomal proteins after synthetic modifications have also been shown to have important roles in gene regulation, a concept formalized as the histone code.[8-10] These relationships have been described in detail in a review elsewhere.[11] The current discussion will focus primarily on the epigenetic mechanisms involved in developmental human β-type globin gene silencing (and hence fetal hemoglobin [HbF] silencing) and the preclinical and potential clinical translational avenues for overcoming this silencing in context of the treatment of inherited β-globin gene disorders.

In all vertebrates that have been studied, a switch from embryonic, or primitive, to definitive hemoglobin production occurs in erythroid cells during development. In humans and old world primates, as well as certain ruminants, an intermediate HbF predominates during mid to late gestational stages and persists at a low level postpartum in definitive erythroid cells after adult hemoglobin predominates (Table I). The details of this switch have been reviewed extensively.[12,13]

As with much of human biology, the ability to identify important regulatory mechanisms that are physiologically relevant is a major challenge requiring robust preclinical models for understanding γ-globin gene silencing in adults and successfully targeting those mechanisms therapeutically. Because of a high degree of evolutionary conservation of gene regulatory mechanisms in erythroid cells, transgenic mice bearing a yeast artificial chromosome (YAC) containing an intact human β-globin gene locus (β-globin YAC) have provided a valuable model system for studying developmental globin gene regulation. The transgenic mouse model also allows for testing the effects of modulating epigenetic processes in the context of whole animal physiology. At the same time, the β-globin YAC mouse model is limited by the fact that the mouse lacks a true analog of the human fetal erythroid compartment, such that the transgenic human γ-globin gene is regulated like the murine embryonic β-type globin genes, which are repressed several orders of magnitude more than the human γ-globin gene in adult humans[14] (Table 1).

Cultured primary human erythroid cells derived from CD34+ progenitors induced to erythroid differentiation provide another powerful model for studying human γ-globin gene silencing.[15,16] The limitations of cultured primary erythroid cells include their limited life span, and the fact that achieving terminal erythroid differentiation while maintaining cell viability is often challenging.

Table I. Developmental stage-specific human and mouse β-type globin gene and corresponding hemoglobin expression patterns

Species	Developmental stage	Predominant β-type globin (hemoglobin)
Mouse	Primitive	β^{H1}, ε^{y} (Hb E) I-III
	Definitive	β^{maj} (Hb β^{maj})
		β^{min} (Hb β^{min})
Human	Primitive	ε (Hb Gower-1, Hb Gower-2)
	Definitive	
	Fetal gestational and	γ (HbF), (Hb Portland-1)
	Postpartum	β (HbA)
		δ (HbA2)

The primate baboon model also has been quite useful given that the developmental β-type globin gene repertoire of the baboon is very similar to humans, including an HbF.[17] Other vertebrate models and cultured cell systems have provided important early insights into epigenetic control of globin gene silencing, but this discussion of preclinical translational studies is directed primarily at the aforementioned models.

Much of the focus of research on developmental γ-globin gene silencing has been on trans-acting transcription factors. The discovery of the quantitative trait locus B-cell lymphoma-leukemia A (*BCL11A*) on chromosome 2p16[18,19] identified this factor as an important regulator of HbF expression. Subsequent studies have shown that BCL11A binds to an intergenic region in the β-globin locus and has a dominant silencing effect on murine embryonic β-type β^{H1} and ε^{y}-globin, as well as human ε- and γ-globin gene expression in β-YAC transgenic mice.[12,20]

Knockdown of BCL11A in cultured primary human adult erythroid cells also results in a significant upregulation of γ-globin gene expression, although the magnitude of this effect is much less than in the β-YAC mouse model.[19] The transcription factor SOX6 also mediates embryonic β^{H1} and ε^{y}-globin gene silencing in the mouse, and it is known to interact with BCL11A.[21,22]

Krüppel-like factor 1(KLF1), originally known as erythroid KLF, EKLF was initially shown to be critical for adult β-globin gene transcription,[23] and to increase the ability of the β-globin promoter to compete with the γ-globin promoter for the enhancer function of the erythroid-specific β-globin locus control region.[24,25] A more direct role of KLF1 in γ-globin gene silencing occurs through its stimulation of *BCL11A* expression.[26,27]