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Switching from fetal to adult hemoglobin

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Abstract

The switch from fetal to adult hemoglobin relies on repression or silencing of the upstream γ -globin gene, but identification of the transcriptional repressors that bind to the sites at which a cluster of naturally occurring variants associated with HPFH (hereditary persistence of fetal hemoglobin) are found has been elusive. A new study provides mechanistic evidence for the direct binding of BCL11A and ZBTB7A, two previously identified γ -globin gene repressors.

In healthy humans, a shift from γ -globin to β -globin gene expression around birth underlies the switch from fetal ($\alpha_2\gamma_2$; HbF) to adult ($\alpha_2\beta_2$; HbA) hemoglobin production, such that by 6 months of age the major hemoglobin is HbA. The hemoglobin switch, however, is not total or irreversible; all adults retain the ability to produce residual levels of HbF (<1% of total hemoglobin)¹. The degree of HbF persistence varies between adults and is largely genetically controlled. Inappropriately high γ -globin gene expression in adults is associated with HPFH, caused by *HBB* cluster deletions or point mutations in the proximal promoter of *HBG1* and *HBG2* (γ -globin genes)². While persistence of high levels of HbF has no clinical consequences in healthy individuals, coinheritance of HPFH with either of the two major β -hemoglobin disorders—sickle cell disease (SCD) or β -thalassemia—alleviates their clinical severity. The point mutations associated with HPFH are characterized as resulting in significant elevation of HbF, which ranges from 10–40% of total hemoglobin in heterozygotes. These mutations are clustered in two regions, located 115 and 200 bp upstream of the transcription start site. They were long postulated to be potential binding sites for γ -globin gene repressors, but identification of the transcription factors binding to these sites has been elusive³. BCL11A and ZBTB7A (also known as LRF) are two recently identified γ -globin gene repressors. It has not been possible to demonstrate direct binding of BCL11A to γ -globin gene promoters, leading to the hypothesis that BCL11A activates the γ -globin genes indirectly via long-range interactions in the *HBB* complex^{4,5}. In this issue, Martyn and colleagues⁶ demonstrate for the first time that BCL11A and ZBTB7A are able to directly bind to the promoter of the γ -globin gene.

Hemoglobin switch and HPFH

The naturally occurring HPFH-associated mutations have been studied since the 1980s³, leading to the model that turning off the γ -globin gene during the hemoglobin switch

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Competing interests

The authors declare no competing interests.

involves two mechanisms: gene competition and autonomous gene silencing of the γ -globin gene¹. The dense clustering of the point mutations associated with HPFH suggests that these are potential binding sites for transcriptional repressors that result in autonomous silencing of the γ -globin gene (Fig. 1). Although the mutations have been shown to alter in vitro binding patterns for a variety of transcription factors with roles in globin gene regulation, there was no consistent theme.

BCL11A and ZBTB7A

BCL11A and ZBTB7A are both C2H2 zinc-finger transcription factors. BCL11A, hitherto unknown to have a role in erythropoiesis, was first implicated as a potential modulator of HbF levels in two genome-wide association studies (GWAS)^{7,8} that showed that SNPs within intron 2 of *BCL11A* correlated most strongly with HbF expression. Functional studies (DNase I hypersensitivity, ChIP-seq and ChIP-qPCR assays) in primary human erythroid progenitor cells and mouse transgenic *lac* reporter assays demonstrated that BCL11A acts as a repressor of γ -globin gene expression, which was effected by SNPs in intron 2 of this gene⁹. Inactivation of *Bcl11a* rescues sickle cell defects in humanized SCD mice¹⁰. Systematic dissection using the CRISPR-Cas9 genome-editing approach further refined the causative variants to an erythroid-specific enhancer in intron 2 of *BCL11A*¹¹, paving the way to use disruption of this site in a genome-editing approach as a therapeutic for reactivation of HbF in SCD and β -thalassemia. Inability to demonstrate direct binding of BCL11A to the γ -globin gene promoters, however, led to the hypothesis that the mechanistic basis for the increased γ -globin gene expression was via long-range interactions of *BCL11A* with *SOX6*, leading to reconfiguration of the *HBB* complex, which increases interaction of the upstream locus-control region (LCR) with the γ -globin gene promoter⁴. ZBTB7A was initially identified as a critical factor in oncogenesis and cellular transformation¹². More recently, it was shown to be a major repressor of the human γ -globin gene¹³.

Here Martyn and colleagues⁶ reversed the strategy by initially screening for transcription factors that bound to the γ -globin gene promoters at the site of the naturally occurring HPFH-associated mutations, using electrophoretic mobility shift assays (EMSAs), demonstrating that BCL11A and ZBTB7A bound in vitro. Using zinc-finger domains of BCL11A and ZBTB7A expressed in COS cells, they demonstrated direct binding of BCL11A to the site at -115 bp with respect to the γ -globin gene transcription start site and ZBTB7A binding to the site at -200 bp. They hypothesize that epitope masking or dynamic binding at a specific time during red blood cell maturation could be responsible for previous failure to demonstrate direct binding of BCL11A to the γ -globin gene promoter. CRISPR-Cas9 editing was used to introduce a new epitope tag system that comprised a tamoxifen-responsive estrogen moiety (ER) and a V5 tag on endogenous BCL11A in HUDEP2 cells. Addition of tamoxifen activates the ER-V5-tagged BCL11A, allowing translocation of the tagged protein to the nucleus, thus avoiding epitope masking. Martyn and colleagues⁶ were able to show that BCL11A bound to the site at -115 bp and ZBTB7A bound to the site at -200 bp in the γ -globin gene promoter (Fig. 1). Interestingly, binding of both BCL11A and ZBTB7A was observed only in clones where the γ -globin gene was expressed, indicating that binding would not be detected when the *HBB* locus is fully silenced. The authors

postulate that this could explain why previous ChIP studies could not show *BCL11A* binding to the γ -globin gene proximal promoters. Introducing HPFH-associated mutations at the sites at –200 and –115 bp resulted in loss of binding activity and increased HbF expression in comparison to wild-type cells, mimicking HPFH. The independent binding sites of *BCL11A* and *ZFTB7A* lend support to an earlier study that showed that *ZFTB7A* and *BCL11A* double-knockout cells had greater increases in HbF than *ZFTB7A* or *BCL11A* single-knockout cells¹³.

What is not clear is whether binding by the two repressors is successive or what order is needed to bring about the full chromatin reconfiguration.

Genetic targets for β -hemoglobinopathies

Compelling evidence from the naturally occurring HPFH-associated mutations shows that elevated HbF levels can alleviate the clinical severity of β -hemoglobinopathies, prompting both pharmacological and genomic approaches for therapeutic HbF reactivation. One genetic approach currently being explored is disruption of the erythroid-specific enhancer of *BCL11A* by CRISPR–Cas9 genome editing¹⁴. However, simulating naturally occurring HPFH-associated mutations may be a more attractive approach, as in a recent proof of principle where deleting 13 bp in the γ -globin gene promoter (a naturally occurring HPFH-associated mutation) in primary human erythroid progenitor cells led to relatively increased HbF expression¹⁵. Martyn and colleagues⁶ used CRISPR–Cas9-mediated genome editing of a human erythroid cell line to recapitulate various naturally occurring HPFH-associated mutations in the γ -globin gene promoter and successfully disrupted binding of the two transcription factors. The findings confirm these sites as potential DNA targets for genome-editing-mediated therapy of β -hemoglobinopathies and provide support for autonomous silencing of the γ -globin gene in hemoglobin switching. □

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Fig. 1 |. Point mutations in γ -globin gene promoters disrupt binding of transcriptional repressors, resulting in elevated fetal hemoglobin levels in adulthood.

BCL11A and ZBTB7A bind to the γ -globin promoter in two sites with clustered mutations in HPFH and inhibit expression. However, individuals with HPFH-associated mutations have a mutated consensus sequence for BCL11A and/or ZBTB7A binding, de-repressing fetal hemoglobin expression during adulthood. The consensus sequences for BCL11A and ZBTB7A binding are shown in red. The HPFH-associated mutations in the cluster (inverted arrows) at -200 bp, from left to right, are c.-202C>T/G, c.-201C>T, c.-197C>T, c.-196C>T and c.-195C>G; the HPFH-associated mutations (upward arrows) in the cluster at -115 bp, from left to right, are c.-117G>A and c.-114C>T/A/G.