GENE REGULATION

Targeting ZNF410 as a potential β-hemoglobinopathy therapy

The nucleosome remodeling and deacetylase (NuRD) complex is a chromatin modifier with a key role in the switch from fetal to adult hemoglobin. In a new study, Vinjamur et al. identify a fetal hemoglobin repressor, ZNF410, which does not directly bind the γ-globin promoter but acts through highly specific regulation of CHD4, a protein subunit of the NuRD complex, thus presenting a potential approach for therapeutic reactivation of fetal hemoglobin.

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The burden of sickle cell disease (SCD) and β-thalassemia is high, affecting several millions of people globally, and is expected to increase in the years to come[1]. Both disorders are caused by mutations in the β-globin gene (*HBB*), yet, despite their apparent genetic simplicity, both display a remarkable spectrum of disease severity and share a common major genetic modifier—fetal (α2γ2) hemoglobin (HbF) levels[2]. The beneficial effects of HbF are lost in both disorders in the first postnatal months after completion of the hemoglobin switch. When the HbF level reaches ~1%, adult hemoglobin becomes predominant, and the diseases are manifested. Therapeutic reactivation of HbF production for these two β-hemoglobin disorders has prompted years of research focused on understanding the fetal–adult hemoglobin switch and how to derepress this switch through pharmacological and genetic or genomic approaches[3]. Genome-wide association studies identified BCL11A as the first key repressor protein found to silence the fetal (γ) globin genes[4], which was followed by the identification of ZBTB7A (also known as LRF)[5]. Each of these repressors binds a cognate recognition site within the γ-globin promoter[6,7]. Clinical trials aimed at downregulating BCL11A through different genetic approaches (lentiviral short hairpin RNA and CRISPR–Cas-9 editing) are currently enrolling participants; this approach shows immense promise in elevating HbF and resolving disease complications[8,9]. However, these 'curative' therapies are currently available to only a fraction of the population affected by SCD and β-thalassemia, even in well-resourced countries. In the near term, a pharmacotherapeutic approach—that is, a drug that can be taken orally—is urgently needed to make therapy available worldwide[10].

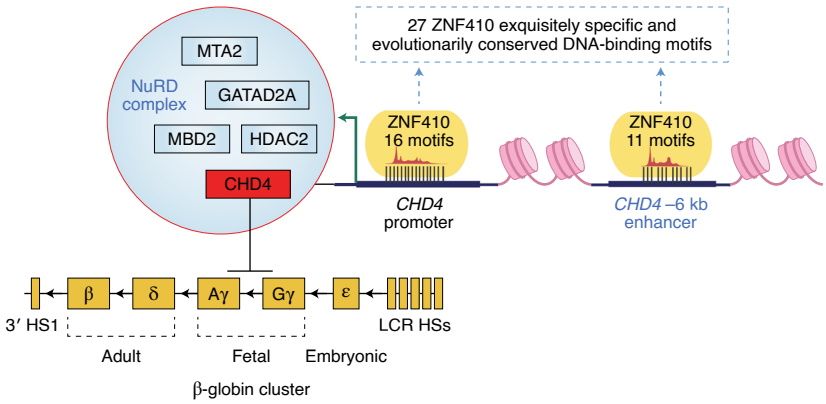

Fig. 1 | ZNF410 transactivation of *CHD4* through 27 evolutionarily conserved DNA-binding motifs. The five zinc fingers of ZNF410 encoded by exons 6–9 regulate CHD4 expression through occupation of *CHD4* chromatin clusters around two sites: the promoter and −6-kilobase enhancer, with 27 evolutionarily conserved motifs. ZNF410 loss due to CRISPR–Cas9-based genome editing disrupts this chromatin occupancy, thus decreasing expression of CHD4, and increasing HbF without affecting erythropoiesis and cell fitness. HS, DNase I–hypersensitive site; LCR, locus control region.

NuRD complex and epigenetic control of the fetal–adult hemoglobin switch

Epigenetic regulation has an important role in the developmental silencing of the γ-globin genes, and the NuRD complex acts as the gateway underlying this regulation. For example, manipulating the DNA methylation status with the competitive DNA methyltransferase inhibitors 5-azacytidine and decitabine leads to HbF elevation in SCD[11]. DNA methylation at the γ-globin locus is recognized by the methylcytosine-binding-domain protein MBD2, which helps recruit the NuRD silencing complex to the locus. The NuRD chromatin-remodeling complex is composed of six different protein-family subunits, with a total of 13 subunit proteins[12,13], including MBD2 and MBD3. This complex also includes two catalytic subunits—the ATP-dependent chromatin remodelers CHD3 and CHD4—as well as the histone deacetylases HDAC1 and HDAC2, both of which have been targeted by HDAC inhibitors as a potential pharmacotherapeutic approach[14]. Although BCL11A and ZBTB7A, the two principal repressor proteins silencing the γ-globin genes, each bind a cognate recognition site within the γ-globin promoter, both factors must physically associate with members of the NuRD corepressor complex to effect their repressive activity, thus emphasizing the essential role of the NuRD complex in hemoglobin switching. Hence, there has been a push to define the mechanisms underlying how the various NuRD subunits interact in producing an active complex for HbF repression and to identify molecular targets for pharmacotherapy.

Recent studies using CRISPR-based saturating mutagenesis in mouse and human primary erythroid culture systems have shown that knockdown of *CHD4*, encoding a NuRD subunit, results in a robust increase in γ-globin expression; these studies have also identified five genes encoding NuRD subunits (*CHD4*, *GATAD2A*, *HDAC2*, *MBD2* and *MTA2*) as essential for HbF repression[13]. Although this step is certainly promising, each of these NuRD subunits displays pleiotropy, regulating various gene expression programs on this chromatin complex, and are thus not desirable molecular targets because of the likelihood of undesirable off-target effects.

ZNF410 regulates HbF through devoted maintenance of CHD4

In this issue, Vinjamur et al.[15] combined a strategy of a CRISPR-based screen using multiple guide RNAs for 1,591 transcription factors and 13 genes of the NuRD complex in the primary human erythroid precursor cell line HUDEP-2, then performed an HbF expression screen. Because HUDEP-2 cells express an adult-type pattern of globins, cells enriched in HbF are potential targets for further investigation. As expected, known HbF regulators, such as *BCL11A* and *ZBTB7A*, showed elevated HbF enrichment scores; genes encoding NuRD-complex subunits, including *CHD4*, *MTA2*, *GATAD2A*, *MBD2* and *HDAC2*, also showed HbF-enrichment scores. Interestingly, the authors observed that single guide RNAs targeting *ZNF410* were also associated with robust HbF induction, but, in contrast to the HbF induction mediated by BCL11A, that mediated by ZNF410 did not compromise cell fitness. *ZNF410* encodes a zinc-finger protein, and exons 6–9 encode a cluster of five C2H2 zinc fingers, which appeared to be sufficient for DNA binding and HbF enrichment without compromising cell fitness. Cleavage under targets and release using nuclease (CUT&RUN) was used to identify ZNF410's targets; it did not occupy any chromatin in the α-globin or β-globin gene clusters, unlike BCL11A and ZBTB7A, which bind the proximal promoter elements at γ-globin (*HBG1* and *HBG2*) genes. Instead, ZNF410 had very restricted chromatin occupancy, with two peaks of densely clustered motifs enriched at the *CHD4* promoter (16 binding motifs) and an enhancer 6 kilobases upstream of *CHD4* (11 binding motifs) (Fig. 1). A contemporaneous study has found similar results regarding the DNA-binding zinc fingers and genomic chromatin occupancy of ZNF410, although with slightly different enrichments and binding motifs at other genes, perhaps owing to the different experimental techniques and approaches used in their study (chromatin immunoprecipitation coupled with high-throughput DNA sequencing, and electrophoretic mobility shift assay)[16]. The chromatin occupancy of ZNF410 at the two upstream elements of *CHD4* was essential for *CHD4* expression, as confirmed by deletion of these elements in HUDEP-2 cells. Additionally, cells in which the upstream element was deleted were competent for further γ-globin induction by BCL11A and ZBTB7A, thus suggesting a distinct mechanism of ZNF410 and CHD4 in HbF regulation.

Clinical implications and relevance

This study has established an HbF-regulatory mechanism involving modulation of the expression of the NuRD subunit CHD4 by ZNF410—a mechanism not previously implicated in globin gene regulation or erythropoiesis. To explore the potential of targeting ZNF410 for therapeutic purposes, its other roles must be investigated. To this end, Vinjamur et al., using HUDEP-2 cells, human erythroid precursor cells, a mouse erythroid cell line (MEL cells) and mouse models, have shown not only that ZNF410 chromatin occupancy is tightly clustered and restricted to the *CHD4* locus in its regulation of HbF, but also that ZNF410 is dispensable in erythropoiesis and hematopoiesis. Deficiency or complete absence of ZNF410 in mice was well tolerated throughout erythropoiesis, hematopoiesis and development, with no evidence of anemia or hemolysis. The focused regulation by ZNF410 with chromatin occupancy at two upstream elements of *CHD4* enhances the prospects of ZNF410 as a small-molecule therapeutic target for HbF induction. ❐

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Competing interests

The authors declare no competing interests.