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Targeting ZNF410 as a potential β -hemoglobinopathy therapy

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Abstract

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

The burden of sickle cell disease (SCD) and β -thalassemia is huge, affecting several millions globally, and is expected to increase in the years to come¹. Both disorders are caused by mutations in the β -globin gene (*HBB*), and yet, despite their apparent genetic simplicity, both disorders display a remarkable spectrum in their disease severity and share a common major genetic modifier – fetal ($\alpha_2\gamma_2$) hemoglobin levels². The beneficial effects of fetal hemoglobin (HbF) are lost in both disorders in first postnatal months with completion of the Hb switch, when HbF level reaches ~1% and adult Hb (HbA) becomes the predominant Hb, and the respective diseases become manifested. Therapeutic re-activation of HbF production for these 2 β -Hb disorders has prompted years of research focused on understanding the fetal-adult Hb switch and how to de-repress this switch using pharmacological and genetic/genomic approaches³. Genome-wide association studies (GWAS) identified BCL11A, as the first key repressor protein for silencing of the fetal (γ) globin genes⁴, later joined by ZBTB7A (LRF)⁵; each binding to a cognate recognition site within the γ -globin promoter^{6,7}. Clinical trials aimed at downregulating BCL11A using 2 different genetic approaches – lentiviral shRNA and CRISPR/Cas-9 editing are currently enrolling and show immense promise in elevating HbF accompanied by resolution of disease complications^{8,9}. However, these “curative” therapies are currently available to only a fraction of the population affected with SCD and β -thalassemia, even in the well-resourced countries. What is urgently needed in the near-term is a pharmacotherapeutic approach, a drug that can be taken orally in order to make it available worldwide¹⁰.

The NuRD complex and epigenetic control of fetal-adult hemoglobin switch

Epigenetic regulation plays an important role in the developmental silencing of the γ -globin genes, and the NuRD complex acts as the gateway underlying this. For example,

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Competing Interests:

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manipulating the DNA methylation status through competitive DNA methyltransferase inhibitors, 5-azacytidine and decitabine, leads to HbF elevation in SCD¹¹. DNA methylation at the γ -globin locus is recognized by the methylcytosine-binding domain (MBD) protein MBD2, which helps to 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, the latter enzymes being targeted by HDAC inhibitors as a potential pharmacotherapeutic approach¹⁴. Although BCL11A and ZBTB7A, the 2 principal repressor proteins for silencing of the γ -globin genes, each bind to a cognate recognition site within the γ -globin promoter, both factors need to physically associate with members of the NuRD corepressor complex, to effect their repressive activity, emphasizing the essential role of the NuRD complex in Hb switching. Hence, the push to define the mechanisms of how the various NuRD subunits interact to produce 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 NuRD subunit *CHD4* results in robust increase in γ -globin expression, and also identified 5 NuRD subunits (*CHD4*, *GATAD2A*, *HDAC2*, *MBD2*, and *MTA2*) as essential for HbF repression¹³. Although this is certainly a promising step, each of these 5 NuRD subunits displays pleiotropy, regulating various gene expression programs on this chromatin complex, and are thus not desirable molecular targets because of likely undesirable off-target effects.

ZNF410 regulates HbF through devoted maintenance of CHD4

Here, 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 HUDEP-2, a primary human erythroid precursor cell line followed by a HbF expression screen. As HUDEP-2 expresses an adult-type pattern of globins, cells that are enriched for HbF represent potential targets for further investigation. As expected, known HbF regulators such as *BCL11A* and *ZBTB7A* (*LRF*), showed elevated HbF enrichment scores; NuRD complex subunits including *CHD4*, *MTA2*, *GATAD2A*, *MBD2*, and *HDAC2* also showed HbF enrichment scores. Interestingly, the authors observed that sgRNAs targeting *ZNF410* were also associated with robust HbF induction, but in contrast to *BCL11A*, *ZNF410*-mediated HbF induction did not compromise cell fitness. *ZNF410* encodes a zinc finger protein, exons 6–9 comprise the cluster of five C2H2 zinc fingers (ZFs) that seemed sufficient for DNA binding and HbF enrichment without compromising cell fitness. CUT&RUN (cleavage under targets and release using nuclease) was utilized to identify *ZNF410*'s targets; it did not occupy any chromatin in the α - or β -globin gene clusters unlike *BCL11A* and *ZBTB7A*, which bind to the proximal promoter elements at γ -globin (*HBG1* and *HBG2*) genes. Instead, *ZNF410* had a very restricted chromatin occupancy, with 2 peaks of densely clustered motifs enriched at the *CHD4* promoter (16 binding motifs) and an enhancer 6 kb upstream of *CHD4* (11 binding motifs) (Fig. 1). A contemporaneous study found similar results on the DNA-binding ZFs 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 (ChIP-seq and EMSA) adopted in their study¹⁶. The chromatin occupancy of ZNF410 at the two upstream elements of *CHD4* was essential for *CHD4* expression, which was confirmed by deletion of these elements in HUDEP-2 cells. Additionally, it was shown that cells where the upstream element was deleted are competent for further γ -globin induction by BCL11A and ZBTB7A, suggesting a distinct mechanism of ZNF410 and CHD4 in HbF regulation.

Clinical implications and relevance

This study has established a fetal hemoglobin regulatory mechanism involving modulation of the NuRD subunit CHD4 expression by ZNF410, hitherto not previously implicated in globin gene regulation or erythropoiesis. To explore the potential of targeting ZNF410 for therapeutic purposes, it is essential to investigate its other roles. To this end, Vinjamur et al. utilized HUDEP-2 cells, human erythroid precursors cells, a mouse erythroid cell line (MEL cells) and mouse models to show that, not only is ZNF410 chromatin occupancy 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 the mouse was well-tolerated throughout erythropoiesis, hematopoiesis, and vertebrate 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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Fig. 1: ZNF410 transactivation of *CHD4* by 27 evolutionary conserved DNA-binding motifs. The 5 zinc-fingers (ZFs) of ZNF410 encoded in exons 6–9 regulate *CHD4* expression through occupation of *CHD4* chromatin clustered around 2 sites: the promoter and –6 kb enhancer, with evolutionarily conserved 27 motifs. ZNF410 loss by CRISPR/Cas9-based genome editing disrupted this chromatin occupancy resulting in the decreased expression of *CHD4*, and elevation of fetal hemoglobin without affecting erythropoiesis and cell fitness.