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Fetal Hemoglobin (HbF) Silencer BCL11A Acts through a Novel DNA-Motif in the Gamma-Globin Promoters, Simplifying the Model for Hemoglobin Switching

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Abstract

Fetal hemoglobin (HbF) level is under strong genetic control. Common genetic variation is associated with modest elevation in HbF levels in adults. Genome-wide association studies led to identification of the regulatory factor BCL11A, which is a major contributor to HbF silencing. Despite clear evidence for

its involvement, how BCL11A acts to repress gamma-globin gene expression has remained unknown. Conflicting data exist in the literature with regard to whether BCL11A binds DNA directly and, if so, the sequence(s) it may recognize. Prior experiments from our laboratory suggested that BCL11A acts at a distance from the gamma-globin gene promoters, but failed to define with confidence sites of occupancy within the beta globin locus. To explore mechanisms by which BCL11A functions, we first devised a cellular assay for rescue of BCL11A-deficient erythroid cells with BCL11A cDNA constructs. This approach established that of the two longer BCL11A isoforms (L and XL), only XL ("eXtraLong") is competent to repress globin expression in cells. XL differs from L by the presence of an alternatively spliced terminal exon encoding three closely spaced zinc-fingers (ZF4-6), implicating this domain in an essential function of the protein. Given that ZFs often mediate sequence-specific DNA-binding, we employed a Protein Binding Microarray platform (PBM) that allows for high-throughput determination of transcription factor binding specificities at high-throughput on an oligonucleotide matrix. We determined that recombinant ZF4-6, as well as BCL11A-XL, binds sequence-specifically to a novel consensus motif with a central core, TGACCA. Two biophysical methods confirmed recognition of this motif by recombinant ZF4-6 with an affinity of ~ 25nM. The motif is present in promoters of all fetal or embryonic-expressed globin genes in human and mouse, and in two copies in each human gamma-globin gene promoter (-119 to -113 and -92 to -86). The motif is altered by single base substitutions in rare HPFH alleles (-117 and -114) and by a microdeletion HPFH (-114 to -102). HPFH-associated single base substitutions greatly impair ZF4-6 binding, therefore strongly implicating a direct role for BCL11A at the gamma-globin gene promoters. However, numerous attempts to localize endogenous BCL11A protein in chromatin of erythroid cells by conventional chromatin immunoprecipitation sequencing (ChIP-seq) proved unsuccessful. To determine if the gamma-globin gene promoters are indeed bound by BCL11A *in vivo* in erythroid cells, we adapted a newly described method for detection of protein occupancy in chromatin (CUT&RUN) at higher resolution and without crosslinking that is commonly used in ChIP-seq. With this method, we detected BCL11A occupancy in the gamma-globin gene promoters in adult erythroid cells, including CD34 cells, and moreover observed that the distal one of the repeated motifs is the predominant binding site *in vivo* . Taken together, these experiments provide persuasive evidence that BCL11A acts locally at the gamma-globin gene promoters to repress transcription and that classical HPFH alleles in the (-117 to -114) region lead to increased HbF due to failure of BCL11A binding. These findings are consistent with a revised model of HbF silencing in which BCL11A acts through direct gamma-globin promoter binding, but do not exclude additional architectural or long-distance roles of BCL11A in HbF silencing. The critical role of the BCL11A DNA-binding motif in mediating HbF repression further supports the rationale for use of genome editing to target this region as therapy of the hemoglobinopathies.

Disclosures

Orkin: *Bioverativ:* Consultancy; *Epizyme Inc.:* Consultancy.

Author notes

*Asterisk with author names denotes non-ASH members.

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