Identification of BCL11A as therapeutic target → Sickle Cell β-Thal.

Known: Persistent high levels of fetal hemoglobin protect against β-Thal.

Goal: Identify SNPs (genes) assoc. w/ HbF. → treatment of SS

Exp: Three GWAS approaches → all 3 similar results → BCL11A

5184 indiv. - genotyped individuals → blood serum

179 indiv. from top to bottom 5% → top

179 indiv. from top to bottom 5% → bottom

GC content - 308,015 markers

SNPs statistically over/under-represented in one pool

Take home: SNPs in 2nd intron of BCL11A assoc. w/ HbF

BCL11A

Chrom 2q15 location

All 3 genes termed in 2nd intron of BCL11A.

1) What is BCL11A?
2) What does it do?
3) Where is it expressed?
4) How is it regulated?
What is it?

BCL11A = Zn finger

Transcription factor

What does it do?

- Knockout - die postnatally + lacks B cells

BCL11A 

Expressed CNS

Fix in erythrocytes: unknown?

What does it do in PBCs? Is it expressed?

1) hbbX

BCL11A

Expression in stage-specific manner: off early, on late

Take home: hbbX expression exhibits inverse correlation to BCL11A exp

What is fix of BCL11A in erythrocytes?

1) ShRNA: 2x ShRNA BCL11A (+ control)

γ-globin Expression

Cell Morph --- : wild-type

human erythroid progenitor cell

Th: Bcl-1 γ-globin

60% - 99% knockdown

Transcriptome --- : wild-type

BCL11A

Strong BCL11A binding

2) Does BCL11A bind Hb locus?
In cells: \( \text{BCL11A} \xrightarrow{?} \text{HbX} \)

Questions: Does BCL11A - HbX in vivo?

Is erythrocyte development normal in absence of BCL11A?

Exp: Create mouse model that deletes BCL11A in erythrocyte lineage + in adult mice

\[ \text{Assess HbX} \rightarrow \text{HbX} > 100 \text{g/dL} \]

\[ \text{Assess transcriptome: only genes impacted} \]

\( \text{EpoL-Cre}^+ \times \text{FloxBC11A} \) (erythrocyte-specific)

Life-inducible Mix Cre x FloxBC11A (adult mice)

Take home: When K.o. BCL11A in erythrocyte lineage

1) HbX skyrockets \( \text{↑} \)
2) RBC dev = normal

Did similar exp in mouse model of SCD

= BCL11A - K.o. in erythrocyte lineage suppressed diseased
Back to the 2nd intron of BCL11A: How is it regulated in the erythrocyte lineage?

DNAase hypersensitive sites

Brain cells known to express high levels

B cells

T cells

( +62kb +56kb +58kb ) + Enhancer

Erythrocytes

Take home: likely enhancer for RBC lineage

Is this region sufficient to drive BCL11A in erythrocytes?

Enhancer? → Lect 2 ?

Is this region necessary for BCL11A expression?

Pre-B cell

Erythrocyte

What sequences are the most critical for BCL11A expression?

Lentiviral 1st round

Enrichment

HbF

Sequence to get sgRNA

Deep sequence