Analysis of lncRNA in the HBS1L-MYB Intergenic Region for Fetal Hemoglobin Expression

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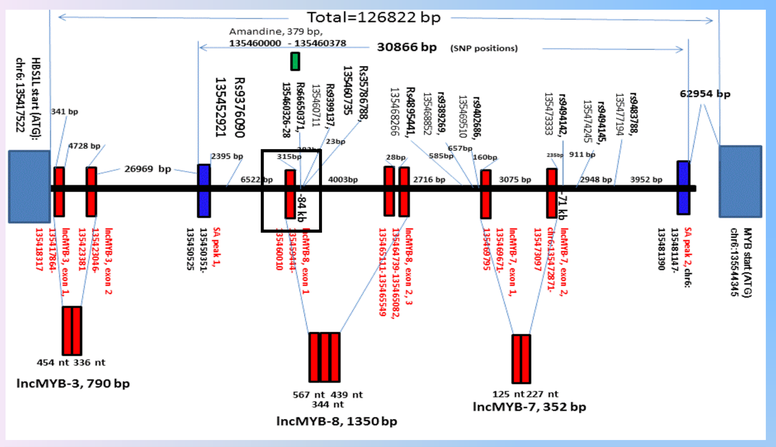
Introduction

Sickle cell disease (SCD) is a point mutation hereditary disease caused by the replacement of glutamic acid by valine at the sixth codon of hemoglobin. This leads to the polymerization of hemoglobin under low oxygen pressure, which can bring about multiple complications including pain, acute chess syndrome, organ malfunction, and increased risk of infection. It is known that the level of fetal hemoglobin is related to the severity of sickle cell anemia and other beta globin disorders. Therefore, raising fetal hemoglobin levels in individuals with sickle cell disease will alleviate the symptoms and ultimately lead to a final cure.

Background

Previous studies have shown three major genetic loci associated with expression of hemoglobin F (Xmn-1HBG2, HMIP-2, and BCL11A). Two genetic blocks within the HMIP-2 locus have been identified and are the focus of this project. There is evidence that these regions may regulate the MYB gene expression with the overexpression of such inhibiting gamma globin gene expression. Despite active transcription at the HMIP region, there are no protein-coded transcripts, suggesting the presence of long non-coding RNA (lncRNA). The characterization of such transcripts can identify them as possible regulators of Hb-F via MYB or some other mechanism. Data mining revealed three lncRNA in this intergenic region (lnc-MYB-3, lncMYB-8. lncMYB-7).



Layout of the HBS1L-MYB intergenic region. The main area of focus for this project was the region surrounding the lncMYB-8. exon 1. Figure not drawn to scale.

Methods and Materials

1. Determine how many transcripts there are in this HBS1L –MYB intergenic region.
2. Determine how these transcript are regulated and how they are related to MYB expression and Hb-F expression.
3. Knockdown the transcript expression to see its influence on MYB and Hb-F gene expression.
4. Delete or mutate the region to see its effects on MYB and Hb-F gene expression.

Results

- Characterization of the -84 kb region, MYB-8 (fig. 1)
- GADPH results verified the quality of cDNA used for testing (fig. 2)
- Gel electrophoresis signals presence of 550 bp transcript (fig. 3)
- Transcripts found upstream and downstream of the 550 bp transcript (fig. 4)
- Tests on CD34+ cells revealed different-sized transcripts, suggesting alternative splicing or possibly heterozygous alleles (fig. 5)
- 379 bp transcript detected in CD34+ cells, band excised and cloned for further inspection (fig. 6)

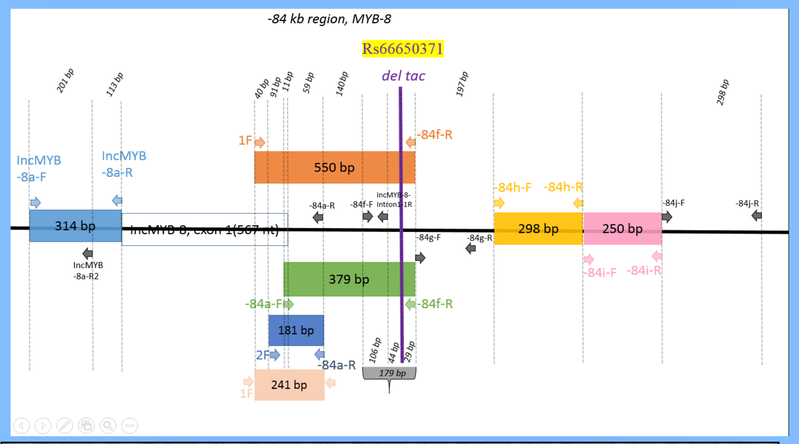


Figure 1: Zoomed in view of the specific region of study. The specifically designed primers are shown and the transcripts that have been identified are shown as colored blocks.

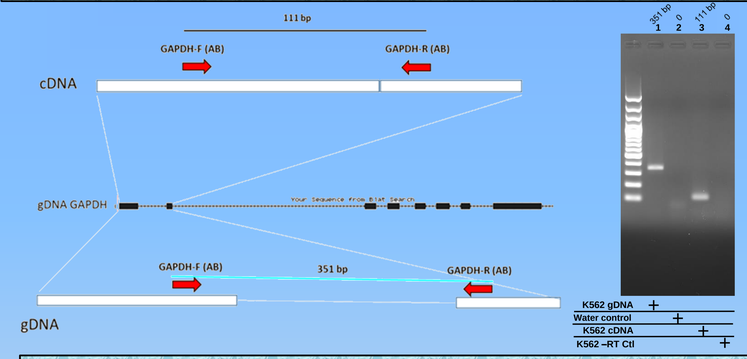


Figure 2: GADPH, a “housekeeping” gene, was detected to ensure that no contamination had occurred. gDNA: 3 µl/lane PCR mix. All other lanes: 15 µl/lane PCR mix.

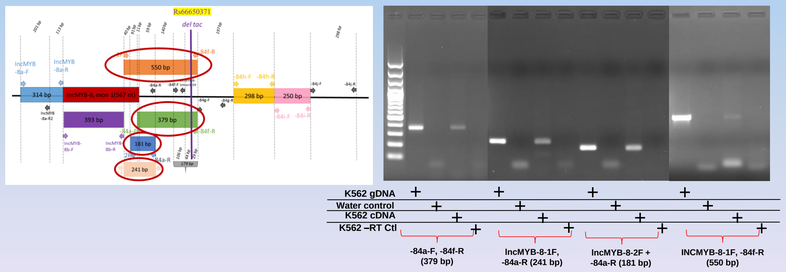


Figure 3: Transcripts of 379, 241, and 181 bp were detected to form a 550 bp transcript. gDNA: 3 µl/lane PCR mix. All other lanes: 15 µl/lane PCR mix.

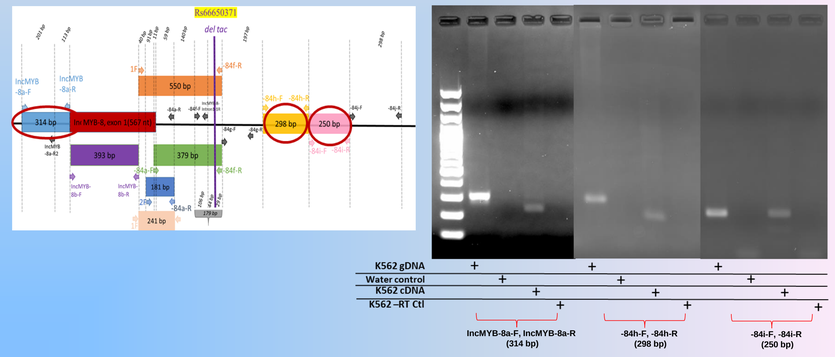


Figure 4: A transcript of 314 bp was detected downstream and transcripts of 298 and 250 bp were detected upstream. gDNA: 3 µl/lane PCR mix. All other lanes: 15 µl/lane PCR mix.

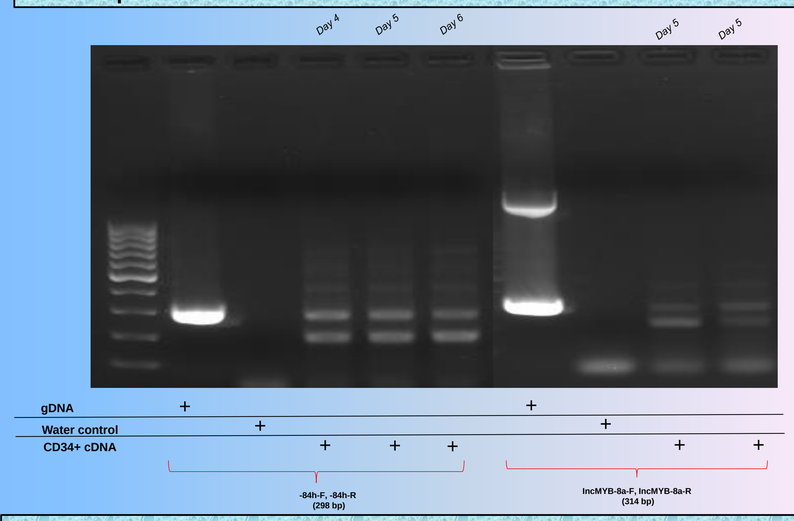


Figure 5: Different sized transcripts of the 298 bp fragment and the 314 bp fragment detected in CD34+ cells. gDNA: 3 µl/lane PCR mix. All other lanes: 15 µl/lane PCR mix.

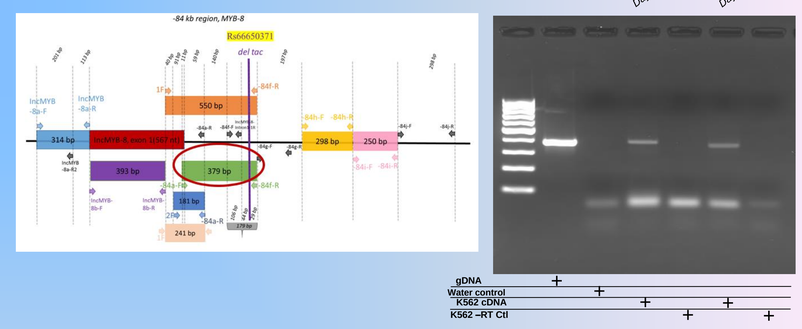


Figure 6: 379 bp transcript detected in CD34+ cells. gDNA: 3 µl/lane PCR mix. All other lanes: 10 µl/lane PCR mix.

Discussion

- Multiple transcripts have been identified, suggesting that this intergenic region is active
- Future work includes conducting RACE to look for the entire transcript and then RNA seq to verify the sequence
- Expression of the transcripts must be further studied and their relation to Myb expression and fetal hemoglobin gene expression must be examined. With this knowledge, the knockdown and knock-out of expression of these transcripts may influence Myb and therefore fetal globin expression.