The Role of LincRNA in B Cells

Amy He
Murre Laboratory

Background

- LincRNA is capped, multi-exonic, tissue specific, and developmentally regulated.
- shRNA makes a tight hairpin turn that silences gene expression by RNA interference
  Virus vector→host genome→transcribed in nucleus→load into RISC→repress mRNA translation/cleave mRNA→target gene silencing
- Research on lincRNA may explain organism diversity.
- Since cell lines can be created with Pre-ProB Cells and ProB Cells, these two types are used in research on lincRNA.

Figure 1. Overall Project Procedure.

Detect lincRNAs that are expressed in B cell development by narrowing down developmentally regulated lincRNAs

Part of the project that is currently focused on:

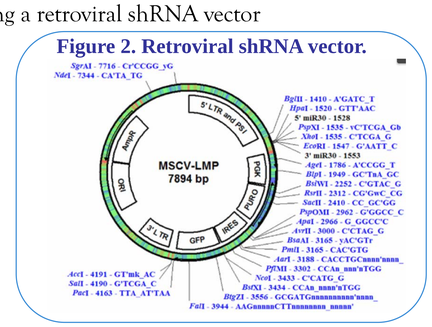
Design several shRNA constructs against each distinct lincRNAs chosen and determine which shRNA is most effective in lincRNA's expression knockdown.

Express effective shRNA in B cells and look at changes in transcript. Examine defects and differentiation in mice's B cells.

Objective

- To shed light on the function of lincRNA by knocking down the expression of lincRNA in B Cells using a retroviral shRNA vector

Figure 2. Retroviral shRNA vector.



Methods

Plasmid Growth

Figure 3. LB/Amp plate.

Allow E.coli bacteria to take up plasmid on a LB/Ampicilin plate.

Pick one colony to grow in LB Broth and Ampicillin. Bacteria grow overnight, and more copies of the plasmid are created.

Figure 4. Bacteria in LB Broth and Amp.

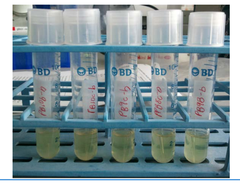
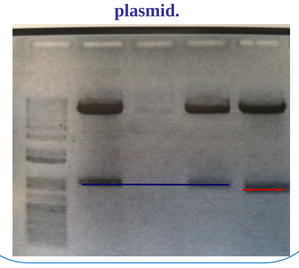


Table 1. Amount of DNA after Mini-Prep.

Purified Plasmid	Amount (ng/μL)
PB9b	639.6
PB9c	772.3
PB10a	550.2
PB10b	730.5
PB10c	580.2
PPB7a	601
PPB7b	512.4
PPB8c	553.8

Figure 5. Electrophoresis on plasmid. Plasmid (blue) is longer than LMP (red). Therefore, shRNA is successfully incorporated in the plasmid.

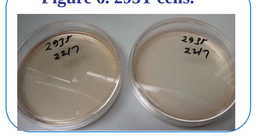


Transfection

Allow 293T, human kidney cells, to take up and grow the plasmid plus packaging plasmid.

Overnight, cells will secrete plasmid-contained virus into the liquid media.

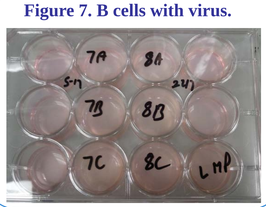
Figure 6. 293T cells.



Spin Infection

Mix B Cells with the liquid media that contains the virus. After centrifuge, allow B Cells to be infected with the plasmid integrated into their genome.

Figure 7. B cells with virus.



FACS

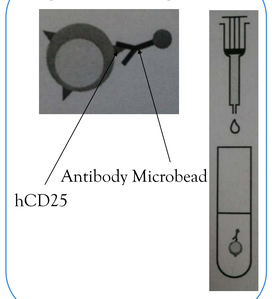
Determine how well B Cells were infected by flow cytometry and expression of CD25.

Figure 8. FACS machine.

Auto-MACS (<80% B Cells infected)

Figure 9. Auto-MACS machine.

Figure 10. Binding to CD25.



The machine uses magnetic microbeads to bind to CD25, separating cells that express CD25 from those that do not as shown in **Figure 10.**

Second Spin Infection (>80% B Cells infected)

Infect the same B Cells again by mixing them with the liquid media that contains virus.

RT-PCR

B Cells are collected, and their RNA are purified to go through RT-PCR. RNA is translated to cDNA. Amplification of cDNA shows small changes in RNA transcript and whether shRNA knockdown was successful. The less transcript detected, the bigger the knockdown and the more effective shRNA was.

Figure 12. RT-PCR Igh1 Amplification Plot.

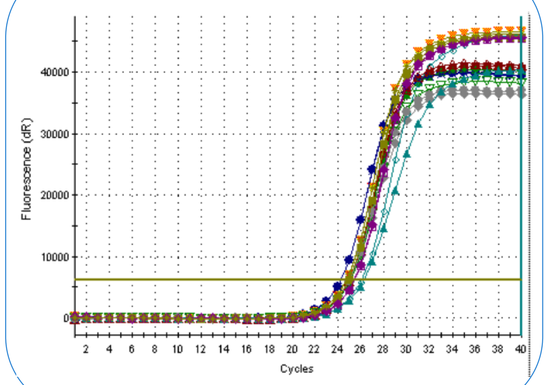
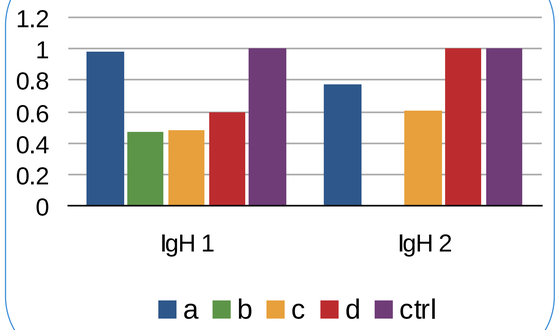
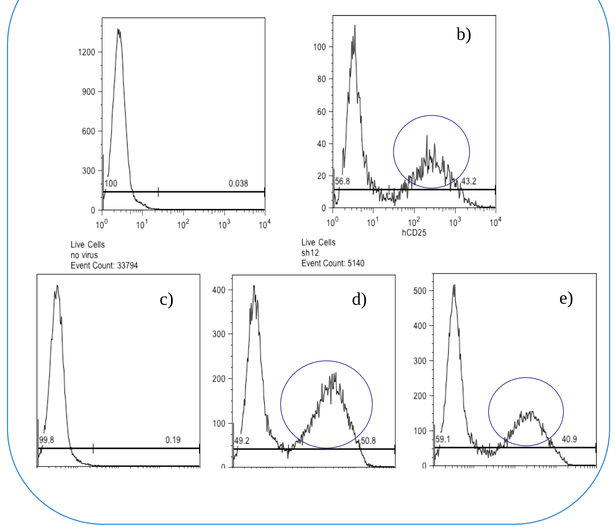


Figure 13. Percent Expressed after shRNA Infection.



Results

Figure 11. FACS data indicating presence of CD25



Discussion and Conclusion

There are variances in the amount of transcript after shRNA infection illustrated in **Figure 12.** Overall, shRNA knockdown was more effective in the IgH1-lincRNA than it was in Igh2-lincRNA as shown in **Figure 13.** 1b and 1c presented the most knockdown; better constructs shall be examined to demonstrate a more efficient knockdown. For IgH2-lincRNA, there was a complication before RT-PCR in 2b, with hardly any RNA found in purification for that sample. Clearly, our shRNA constructs against IgH2-lincRNA were ineffective. In future projects, new constructs will be created to successfully knockdown the expression in IgH2. After the most effective shRNAs are determined for each lincRNA, changes and differentiation in B Cells can be analyzed.

Acknowledgements

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