Identification of microRNAs that Regulate Activation through a PTBP1-Dependent Process in Jurkat cells

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Under the Direction of

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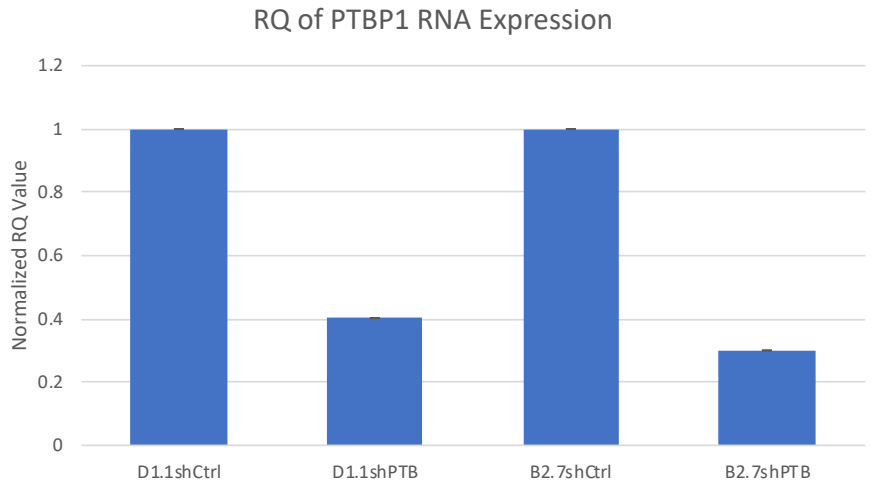
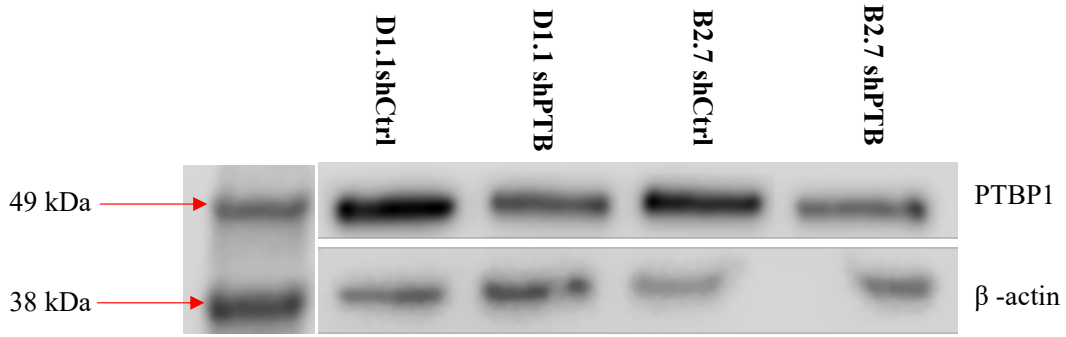
Purpose and hypothesis

- Purpose: determine whether there are regulatory microRNA mechanisms impacting T cell activation and potentially CD40L through a PTBP1-dependent mechanism
- 1. If a microRNA is found to regulate T cell activation through a PTBP1-dependent process, there will be different expression patterns between the D1.1 and B2.7 cell lines
  - D1.1 = more significant role in activated T cells, B2.7 = non-activated or transition to becoming activated
- 2. If a microRNA is found to regulate CD40L expression, this microRNA will be found in D1.1 and not B2.7 cells

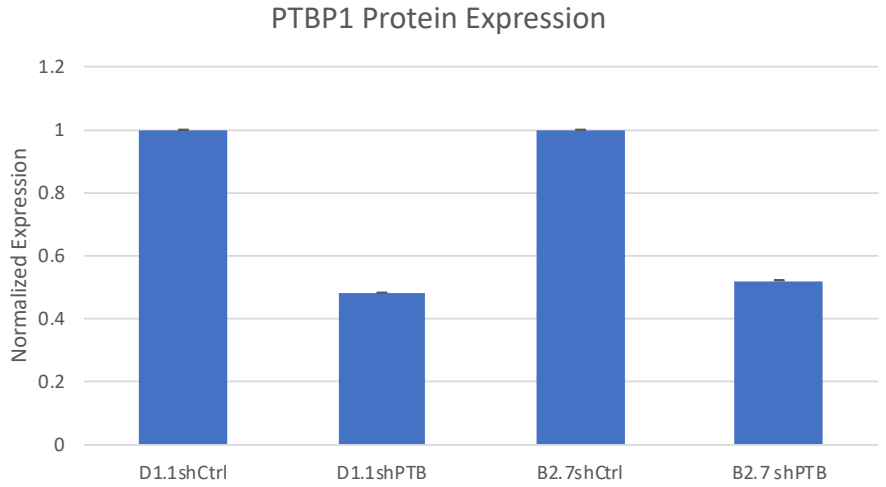
PTBP1 RNA and protein are downregulated in cells expressing shPTB



PTBP1 RNA expression is downregulated in cells expressing shPTB. After isolating RNA and generating cDNA, qPCR was conducted using the QuantStudio™ system to quantify PTBP1 expression at the RNA level for D1.1shCtrl, D1.1shPTB, B2.7shCtrl, and B2.7shPTB samples. RQ values were normalized to the shCtrl samples and human RPLPO was used as an endogenous control. N =2.

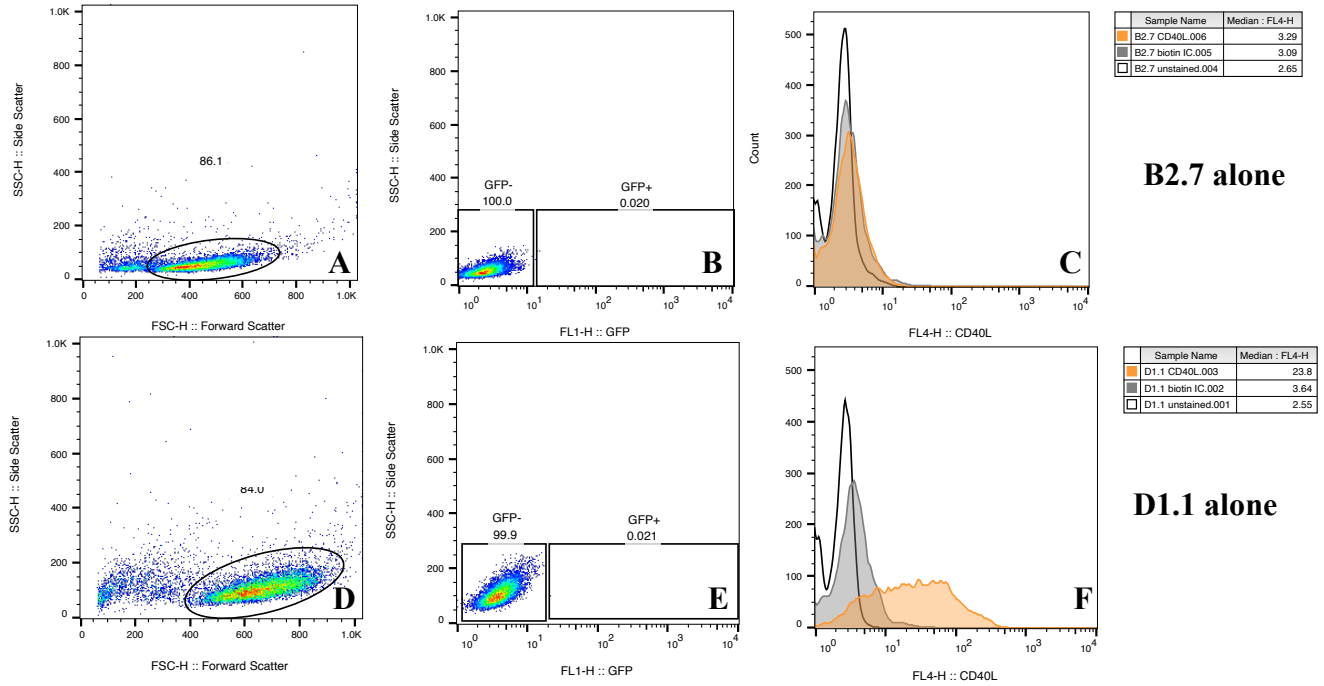


PTBP1 protein is downregulated in cells expressing shPTB. The above western blot images display the results of the blot developed using an antibody against PTBP1, followed by the loading control (β-actin). SeeBlue Plus2 Pre-stained Protein Standard was used. Results were normalized to β-actin expression.



Downregulation of PTBP1 protein in cells expressing shPTB. After isolating protein, a western blot was used to measure the PTBP1 protein expression for D1.1shCtrl, D1.1shPTB, B2.7shCtrl, and B2.7shPTB. Results were normalized to β-actin. All results were normalized to the shCtrl samples, which have a relative expression of 1.00. N = 2.

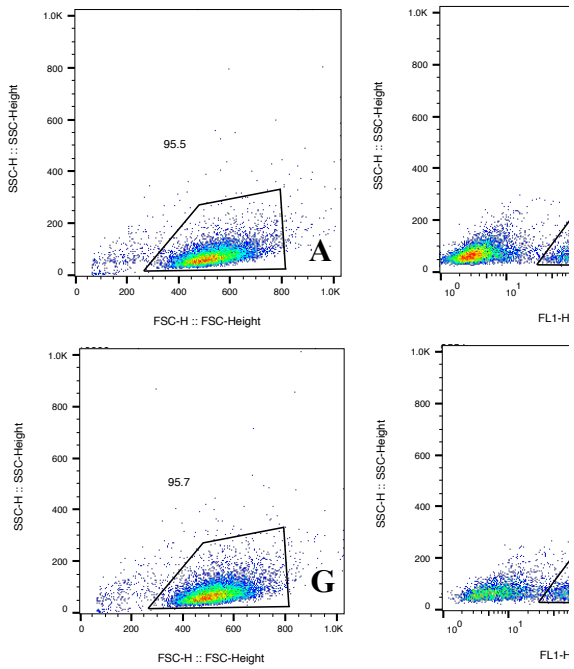
D1.1 cell line expresses high levels of CD40L

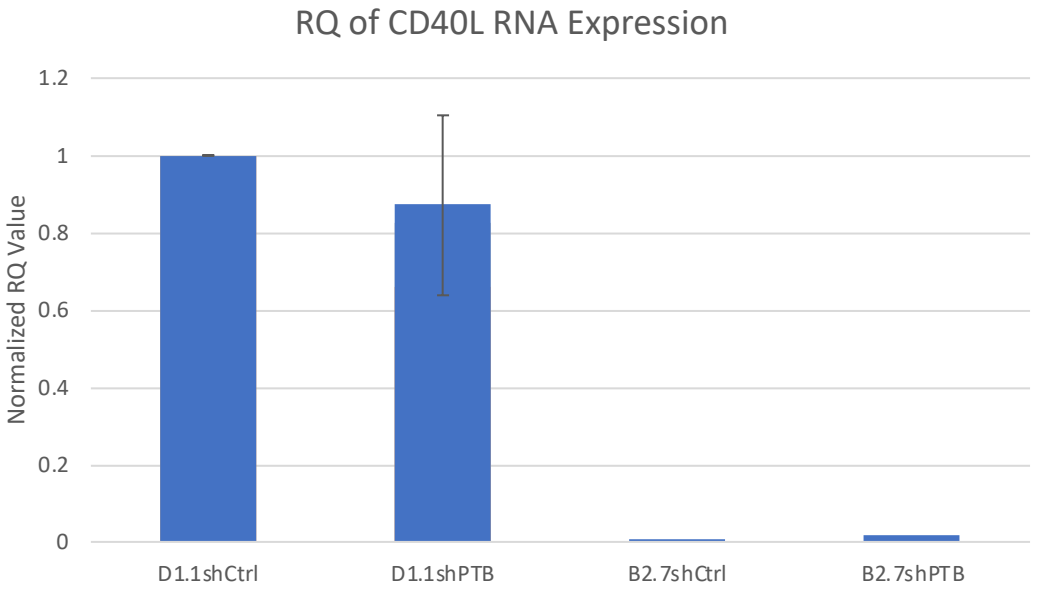


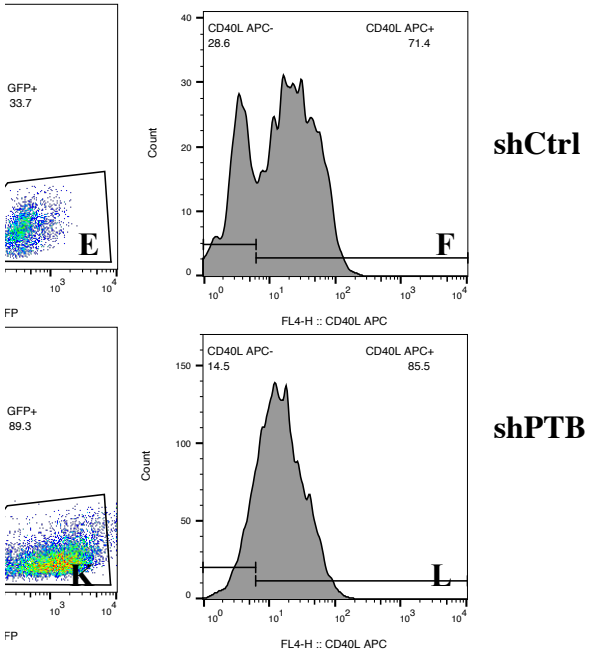
D1.1 cell line expresses high levels of CD40L. Using the FACSCalibur, flow cytometry was used to analyze surface CD40L expression after incubating the cells with an antibody against CD40L. Total live cells, GFP+ and GFP- cells, and median fluorescence intensities (MFI) of cells expressing CD40L, biotin (negative control), and unstained cells for B2.7 alone and D1.1 alone. A and D represent total live cells, B and E represent the GFP+ and GFP- populations, and C and F represent the median fluorescence intensities for cells expressing CD40L, biotin, and unstained cells. A – C represent B2.7 alone, while D1.1 alone is represented by D – F.

Downregulation of PTBP1 does not significantly affect the expression of CD40L in Jurkat D1.1

B2.7 **D1.1**





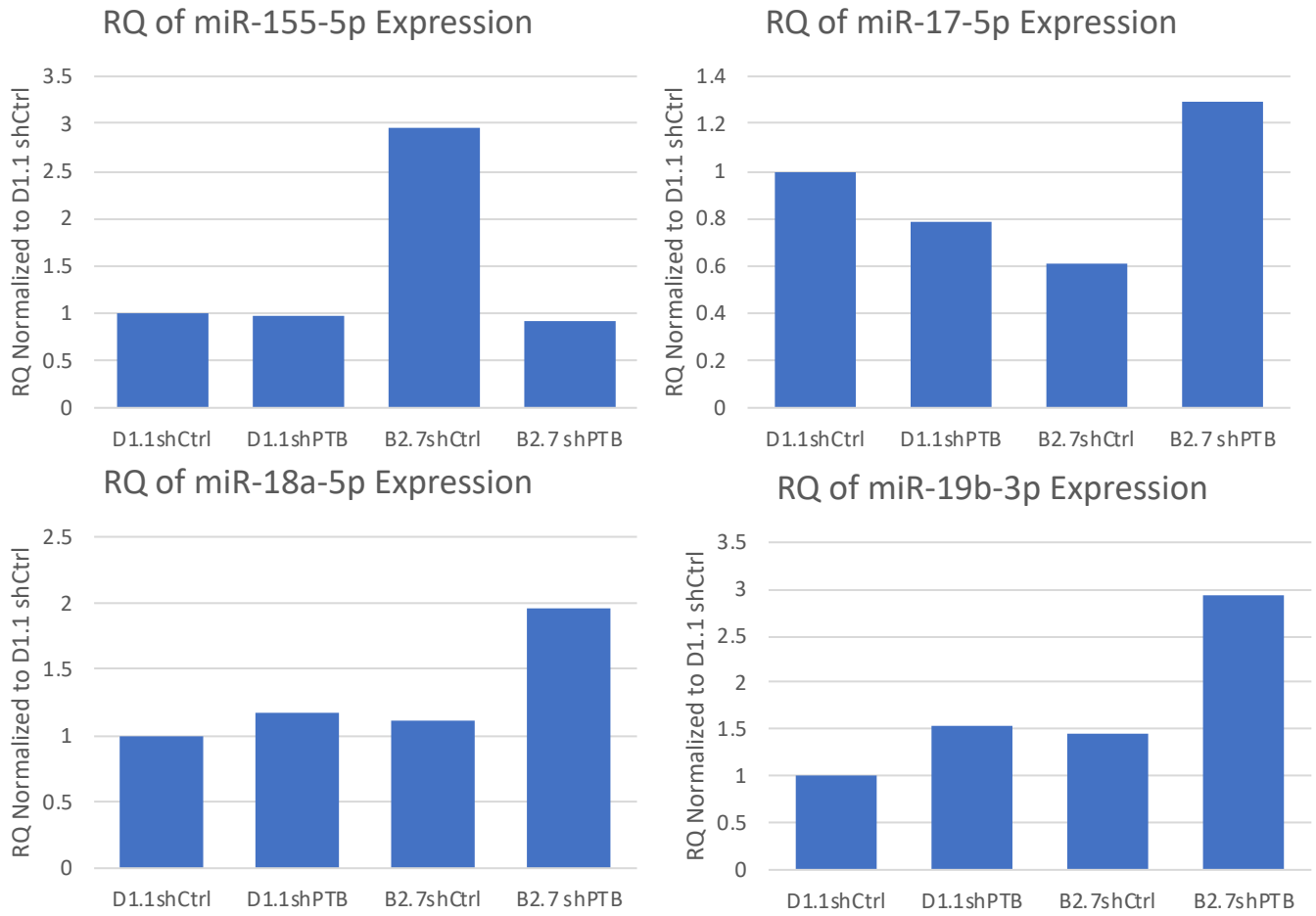


Reducing PTBP1 does not appear to s
surface CD40L expression in GFP+ popul
CD40L APC+ cells for each sample are s
GFP+ CD40L APC- and CD40L APC+ p
The MFI for D1.1shCtrl is 16.1, while the

Downregulation of PTBP1 does not result in dramatically decreased CD40L RNA levels. After isolating RNA and generating cDNA, qPCR was conducted using the QuantStudio™ system to quantify CD40L expression at the RNA level for D1.1shCtrl, D1.1shPTB, B2.7shCtrl, and B2.7shPTB samples. RQ values were normalized to shCtrl samples and human RPLPO was used as an endogenous control. N = 3 for D1.1shCtrl and D1.1shPTB.

libur, flow cytometry was used to analyze
'P+ cells, and total GFP+ CD40L APC- and
FP+ population. C, F, I, and L represent the
2.7shPTB, and J – L represent D1.1shPTB.

Greatest expression of various microRNAs in non-activated phenotype



Greatest expression of various microRNAs in non-activated phenotype. MicroRNAs were isolated and cDNA was generated, followed by qPCR to quantitate expression of various microRNAs. Results were normalized to the endogenous control, miR-26a-5p. RQ values were normalized to D1.1shCtrl.

Conclusion

- In diseases like lupus, miR-155-5p deficiency has been shown to decrease autoantibody levels and symptoms characteristic of the disease (Leiss et al., 2017)
  - Possible that downregulating particular microRNAs can lessen pathology and symptoms of various autoimmune disorders
- Through studying microRNAs and their expression, it will be possible to create a fuller picture of potential microRNAs controlling T cell activation through a PTBP1-mediated process, especially in cases of immune abnormalities

Acknowledgement

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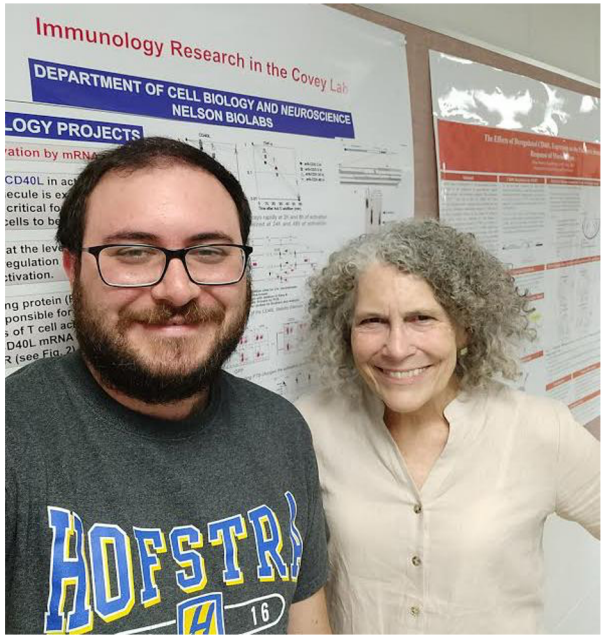
- Dr. Usha Ganapathi, Research Associate
- Diego Prado De Maio, PhD Candidate
- Nicole Rothstein, Undergraduate



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