Rapamycin increases the efficiency of lentiviral transduction

Ann Liu, Cathy X. Wang, Elena A. Federzoni Ph.D., Bruce E. Torbett Ph.D.
The Scripps Research Institute, Department of Molecular and Experimental Medicine, La Jolla, California

Introduction

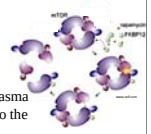
The Life Sciences Summer Institute (LSSI) connects high school students to San Diego's Sciences Industry since 2005. Students one-week pre-internship "boot camp" training followed by 7-weeks of paid research work experience.

Background

- Stem cells are very resistant to transduction (the incorporation of new genes into a cell's DNA, in this case via lentiviral vectors) – they are hard to infect.

How can we increase the efficiency of transduction?

- Treat cells with **rapamycin** – inhibitor of mTOR
  - Preserves stem cells, increases amount of virus entering and integrating into the cell.



How does rapamycin work?

- We already know that rapamycin increases the amount of integration.
- We are testing the step where the virus gets into the cell through the plasma membrane, but before the contents of the virus particle are dumped into the cytoplasm.
- Correlation between how much virus enters the cell and how much is integrated

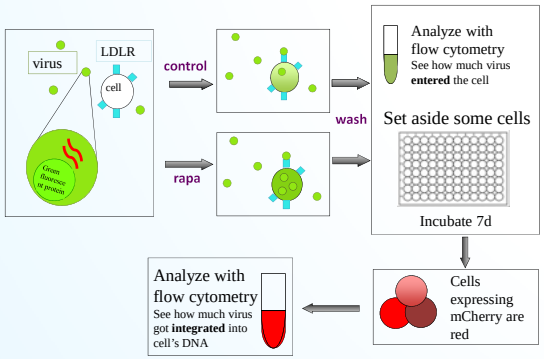
What assay can we use to look at how much virus enters the cell?

Methods

- GFP-Vpr Internalization assay

Cell line model: K562 – characteristics similar to that of true stem cells

- First, we treat the cells with either rapamycin or DMSO (control). Cells with rapamycin should allow more virus to enter the cell and integrate into its DNA.
- Add lentivirus containing green fluorescent protein (GFP) and a gene coding for mCherry – a red fluorescent protein
- The GFP will show us virus entry; the mCherry will show us integration

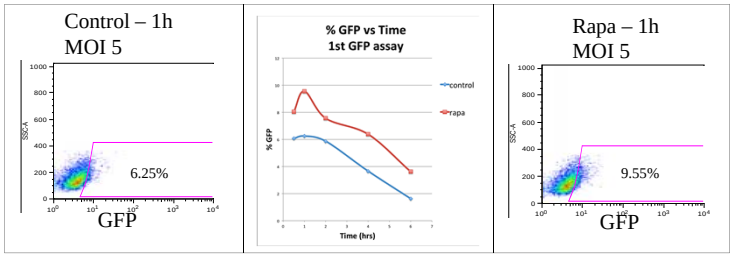


- We can see how much virus enters the cell by using flow cytometry to look at how green the cells are; if they are greener, then more virus entered.
- We can see how much virus is integrated into the cell by looking at how red the cells are.

Results

GFP-Vpr internalization assay, MOI – 5

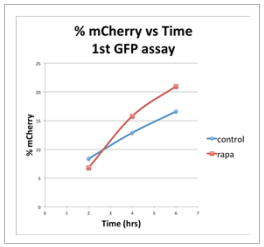
How much virus entered?



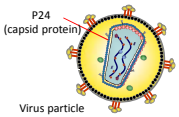
- Cells within pink gate are GFP positive – virus entered these cells
- Population is very small – only about 9% of cells at most display fluorescence
  - *Assay does not efficiently show entry of virus*
- Number of cells with virus is greater when rapamycin is added.

How much virus got integrated?

- At the most, 21% of cells are mCherry positive – are expressing the gene from the lentiviral vector
- Percentage of cells that were transduced increases with time



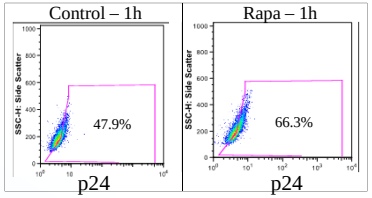
- More cells are transduced with the addition of rapamycin



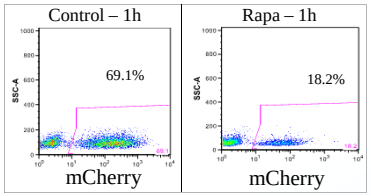
P24 staining assay

- The procedure is the same as the GFP-Vpr assay, except that instead of looking for GFP, we permeablize the cells, allowing the antibodies to go into the cell and bind to the p24 proteins in the vesicles and cytoplasm.

How much virus entered?



How much virus got integrated?



- Cells within pink gate are p24 positive – virus entered these cells

Conclusions

GFP-Vpr internalization assay

- GFP-Vpr assays worked – populations of GFP and mCherry positive cells were detected – however, the numbers were very low.
- *This assay is not efficient.*
  - Applying this assay to true stem cells most likely would not produce results; stem cells are much harder to transduce.

Why were the levels of GFP so low?

- Number of fluorescent proteins per cell too low – flow cytometer not sensitive enough to detect.
- Solution: Raise MOI higher (add more virus), but this would be extremely inefficient and costly.

P24 staining assay

- P24 staining assay worked – populations of p24 and mCherry positive cells were detected – and the numbers are high
- Can be used to observe how much virus enters the cell (levels of p24) and how much virus is integrated and expressed (levels of mCherry)
- *This assay is efficient* and can be applied to true stem cells

Applications

- Efficient gene delivery to hematopoetic stem cells
- Due to increased gene delivery, easily alter the genomes of stem cells
- Potentially allows the use of gene therapy to cure genetic diseases and cancer

Personal Statement

The summer I spent interning at the Torbett lab taught me more than just lab techniques and facts; I got a taste of the passion a scientist has for his/her work and experienced the lifestyle of a researcher.

Acknowledgements

I would like to thank CIRM for funding, my mentors Cathy Wang and Elena Federzoni, our PI Bruce Torbett, the program coordinator Suzanne Russell, my fellow intern Erik Owen, all members of the Torbett lab, all of my other fellow interns, and finally, my family.