Establishing a PU.1 Conditional System

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Introduction

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

Personal Statement: Research is not just trying to answer questions, but it is trying to perpetually discover new questions in order to solve problems that might not be realized yet.

Background Information on Myeloid Cell Development:

- The transcription factor PU.1 causes the differentiation of hematopoietic stem cells (HSCs) into different types of blood cells, specifically, into myeloid cells
- In the absence of PU.1, HSCs have limited to no differentiation causing impaired immune system function, unhealthy red blood cells, and a high risk of cancer
- We need a PU.1 modulating system in order to study how PU.1 affects progenitor blood cells in the early stages of hematopoiesis in order to further study how we might be able to cure diseases like Acute Myeloid Leukemia (AML)

Hypothesis

Does the PU.1_FKBP protein complex become activated in the presence of the small molecule Shield1 causing myeloid cell differentiation and deactivate in the absence of Shield1 in order to create a PU.1 regulatable system?

Methods

PU.1 Null Expression

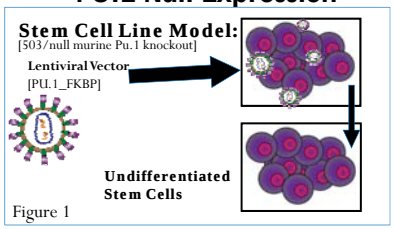


Figure 1. Destabilization Domain FKBP "deactivates" PU.1 by causing it to misfold. PU.1 is not expressed in cells causing them to stay undifferentiated.

Active PU.1_FKBP + Shield1 Expression

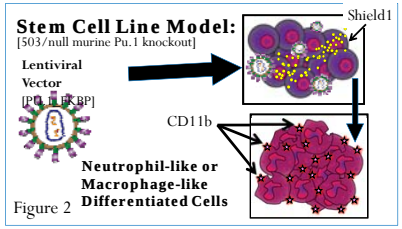


Figure 2. PU.1_FKBP complex is correctly refolded by the stabilizing molecule "Shield1". PU.1 acts as functional transcription factor. Presence of active PU.1 causes HSCs to differentiate and for membrane proteins like CD11b to appear which can be detected through antibody staining.

Results

Does PU.1_FKBP + Shield1 promote active Pu.1 and regulate myeloid cell development?

PU.1_FKBP

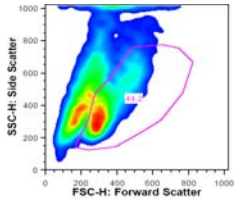


Figure 3. 44.2% of cells were viable in the 503 PU.1_FKBP sample analyzed using flow cytometry.

PU.1_FKBP + Shield1

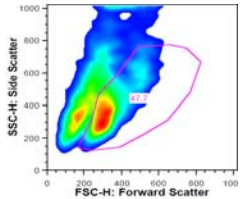
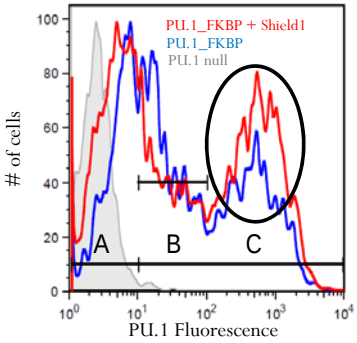


Figure 4. 47.7% of cells were viable in the 503 PU.1_FKBP sample treated with Shield1.

PU.1_FKBP versus PU.1_FKBP + Shield1 Overlay



Analysis:

- Since the population of PU.1_FKBP is present in groups "B" and "C", that means that PU.1 was active in those cells.
- System does not function properly since PU.1 is not completely "deactivated" in the absence of Shield1
- Shield1 successfully increased the amount of cells expressing an active form of PU.1 (Graph 1 C)

Graph 1. Overlay of PU.1_FKBP with and without Shield1.
A) Population of cells not exhibiting CD11b
B) Population of cells with some CD11b present
C) Population of cells expressing large amounts of CD11b

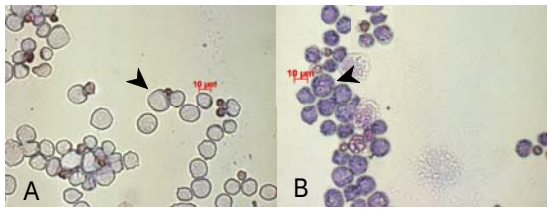


Figure 5. 503/null murine Pu.1 knockout versus 503 cells with PU.1_FKBP added. A) Undifferentiated stem cells, mostly granule-free. B) 503 cells have begun to differentiate, granules are developing inside and the cells are on average, larger than the cells in (A).

Conclusions

Overall, the system is currently unable to function to be able to be used as a tool for studying the role of PU.1 in the early stages of hematopoiesis because it cannot effectively regulate the amount of active PU.1 in the cell at a certain time.

Potential Reasons for why PU.1_FKBP was active in the absence of Shield1

1. **Overexpression of PU.1:**
   - A chaperone protein could possibly be refolding excess inactive PU.1_FKBP complexes to activate PU.1
   - **To solve:** use less virus to decrease the amount of PU.1_FKBP
2. **FKBP is ineffecient:**
   - FKBP could fail to misfold the tertiary structure of PU.1.
   - **To solve:** could use a more efficient destabilzation domain

Applications for Future Use

Once the system is functional, we will be able to:

- Track quantity of PU.1 against time
- Take "snapshots" of PU.1 in action during the early stages of hematopoiesis
- Eventually cure diseases like [Acute Myeloid] Leukemia
- Apply the system to study other transcription factors

Personal Conclusions

My internship taught me to:

- Take proper care of cell lines in culture
- Prepare samples for flow cytometry, and analyze the data obtained
- Appreciate the potential viruses have as a tool for gene delivery and even gene therapy
- and finally, it taught me that I want to be a researcher in the future!

Acknowledgements

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