Optimization of Induction Conditions for Expression of C-Chemokine Receptor 7 (CCR7) Antagonist 8-83 Using the Glutathione S-Transferase (PGEx4T)

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BACKGROUND

T cell acute lymphoblastic leukemia (T-ALL) is rare compared to B-Cell Leukemia; and it tends to have a worse prognosis rate: the T-cell variant accounts for about 15% to 25% of ALL in children. Children and adolescents diagnosed with T cell acute lymphoblastic leukemia are often treated with cranial radiotherapy and/or chemotherapy injected into the cerebrospinal fluid (in the spine) to reduce the risk of central nervous system (CNS) relapse. Due to the T-cell's ability to migrate into the CNS, T-ALL has the capability to "hide" from general chemotherapy, causing a 90% relapse rate within 5 years of diagnoses. Chemotherapy targeted at the central nervous system has the ability to successfully treat T-ALL at the cost of severe defects to the patient. This is because chemotherapy is both the useful and the hazardous; it is essentially like drinking poison to clear your body of cancer cells. The other form of killing off cancer cells is by radiation treatments, in which the patient's cells are "burned" or "broken". Still, both treatments destroy the healthy cells that the patient needs. Although these methods increase long-term survival, the drastic, abiding side effects of these leukemia cancer treatments can include: lowered IQ, slowed learning, stunted growth, more cancer, and more brain tumors.

HYPOTHESIS

By inducing our cells in different conditions, we expect to see different amounts of protein expressed within them.

Figure 1: "*Leukemia Rates In Children And Young Adults, USA, 2005-2009*". Studying Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), and Chronic Lymphocytic Leukemia (CLL).

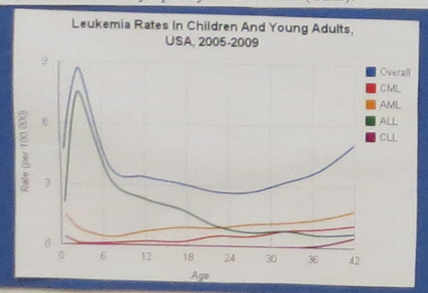
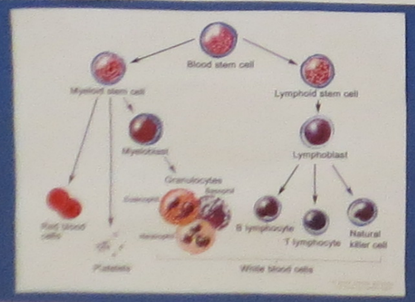
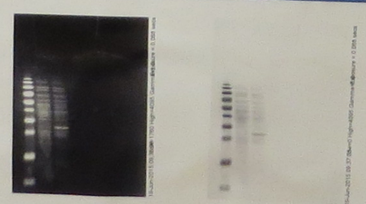


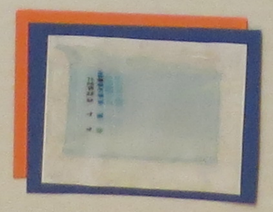
Figure 2. Blood cell development. Different blood and immune cell lineages, including T- and B-lymphocytes, differentiate from a common blood stem cell.



MATERIALS

- Isopropyl β-D-1-thiogalactopyranoside (IPTG)
- BL21 Competent E. coli bacterial strain
- DH5 Alpha E. coli bacterial strain
- SDS-PAGE Analysis Equipment
- Gel Dock XR Imaging Camera
- Lysogeny broth (LB) Media
- 4x Tris Cl/SDS pH 8.8
- 4x Tris Cl/SDS pH 6.8
- 1x Tris/Glycine/SDS
- Spectrophotometer
- Centrifuge
- Incubator
- Shaker





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Methods

We are going to stop the migration of the T cells into the central nervous system by creating an antagonist to the ligand which carries the instructions for the cell to move. The instructions are taken by C-Chemokine receptor 7 (CCR7). By doing this, chemotherapy can have an easier time reaching the T cells and killing the cells off where they are most vulnerable. By finding the optimal conditions for producing the antagonist 8-83, we will be able to purify vast amounts of protein. If the protein is produced in sizeable amounts, this purified protein can then be used as an antagonist for Chemokine (C-C motif) ligand 19 (CCL19). CCL19 is the ligand that binds to CCR7 and directs the cell in its migration. We can purify the protein that is expressed in bacterial cells by lysing them open and removing the 8-83. It would be very impactful to patients who suffer from leukemia, their families, and the medical field if we could optimize growth conditions for 8-83. The higher efficiency in protein production would lead to the CCL19 antagonist protein creation. Generating these large amounts of 8-83 will help further research development in order to create the antagonist. The more rapid the research the sooner we can stop the migration of T cells in these patients and help cure them without harming them even more. To find the optimal circumstances we will be testing four different variables which are temperature of induction, speed of induction, speed of the shaker, and the time of induction.

First, we will be testing the temperature of induction. The different temperatures are 37 degrees Celsius (control), 16 degrees Celsius, and 42 degrees Celsius.

Second is the speed of induction, which we will be slowly adding bit by bit of IPTG as the time period passes. We will induce .4 mM over the periods of 3, 4, and 5 hours.

Next, we will be testing the speed of the shaker. These speeds will be 120 and 300 RPMs (the control is 250 RPMs).

The final variable is the time that the cells are inducted in their colony growth. We will be inducing at the optical densities (OD) of .4 nm, 1.00 nm, and 1.5 nm.

After each of these conditions are tested we can identify to see how the protein was expressed by running a Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE). We can look at this gel and find the band that is supposed to be our expressed gel. The brighter the band the greater the expression in the cells.

Discussion Questions

Q: What will happen if the protein, in any of the selected circumstances, isn't expressed?

A: We will record the results and test it again. If we receive the same result after multiple experiments of the same variable then we will move on to the next condition.

Q: What happens if we do succeed in finding a new way to express more 8-83?

A: If we accomplish our goal we can be able to produce our own 8-83 and purify it. After purification we can use it for being the antagonist to CCL19 and stopping the migration of the T cells into the CNS.

Q: What equipment flaws will be expected?

A: We only have one shaker in our lab that is able for use, and with all the other bacteria that needs to be in there at the normal protocol 250 RPMs it will be difficult to test our different RPM levels when other people need to use the shaker.

REFERENCES

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