Purifying Antagonists 8-83 for Ligand CCR19 Using a Biotinylated Tag

LAB 4

By Ivan Contreras, Cecelia Rocha, Colin Knight, Olga Soto and Dr. Charlotte Vines

Background

T cell acute lymphoblastic leukemia is characterized by a large amount of (greater than 50,000/µl) white blood cells. The normal human body has anywhere from 5,000-10,000 white blood cells per microliter. Acute lymphoblastic leukemia will strike about 3000 children in 2015. Its main victims are male boys from the ages of eight to nine years old. T cell acute lymphoblastic leukemia occurs in 10-15% of these children. It is less common than B-cell lymphoblastic leukemia but can cause be more difficult to cure. One problem is that T-cells have the ability to move into the central nervous system and once they get there they "hide" from the chemotherapy. If the T-cell acute lymphoblastic leukemia cells enter the central nervous system there is a 90% chance that it will come back

Within five years. To treat T cell acute lymphoblastic leukemia scientists inject chemotherapy into the central nervous system or try to kill the tumors with radiation. 66% people who are treated with chemotherapy get brain tumors, permanent brain damage, and stunted growth within being treated with chemotherapy for two to three years. Instead of instead of trying to kill the leukemia by injecting chemotherapy into the central nervous system, scientists are trying different approaches to kill this disease. The need is to eliminate and to inject chemotherapy into the central nervous system, by stopping the movement of the leukemia into the central nervous system via the Blood/brain barrier. This is to be accomplished by using the antagonist, 8-83, to the ligand CCR19, which directs passage of the leukemia into the brain.

In order to create the antagonist 8-83, which acts as a blockade to the ligand CCL19, the protein must be injected in a pure form. Once 8-83 protein is blocking the ligand, No leukemia cells will be able to breach the blood brain barrier, thus keeping the leukemia from hiding in the brain. The only Side effects being temporarily reduced immune responses while the medication is in effect, as well as slight irritation and itching.

Hypothesis

Purifying antagonist 8-83 with biotinylation will allow for the blocking of CCL19 to CCR7, preventing migration of T-ALL to the brain.

Equipment

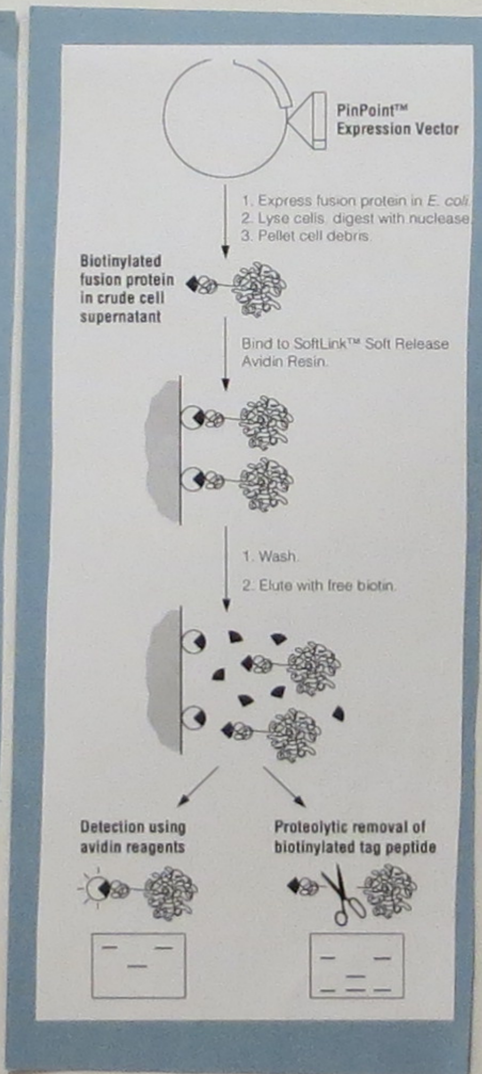
- Carbenicillin
- IPTG
- Monomeric Avidin
- Biotin
- SDS-PAGE analysis

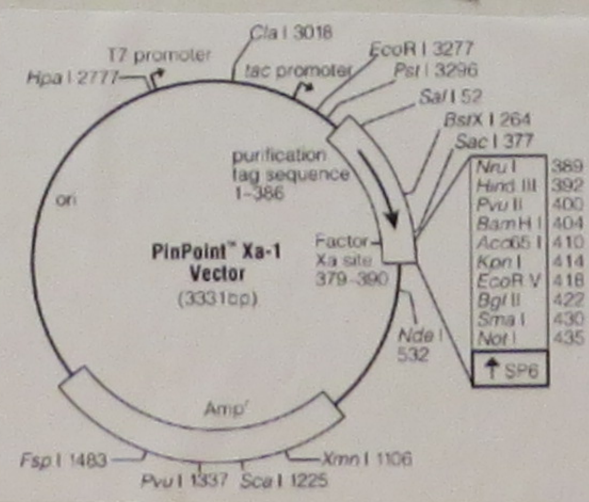
Tools

- Incubator shaker
- Incubator
- Water Bath
- Vortex
- Centrifuge

Methods

1. grow bacteria that contain the vector (encodes the 8-83)
2. Once the bacteria have reached an optical density of .8 OD at 600nm, they will be fractionated by SDS-PAGE to ensure that they express the protein 8-83 within them
3. Once this is confirmed, they will be grown up in 5ml cultures containing both biotin (100ul) and carbenicillin (5ul)
   -Labeled 1,2, and 3
   -This is so that the protein can be separated later by biotinylation.
4. The three original cultures are then separated into six spate tubes of 50ml Lb also containing biotin (1 ul) and Carbenicillin (50 ul). Each 50 ml tube is then mixed with .5 ul of the original 3 cultures with two tubes being created for each culture.
   -Label each 1.1,1.2,2.1,2.2 (this will help determine which one produced the result)
5. Tubes shaken and induced with IPTG
6. Separation of 1.5 ml tubes from each of the six current cultures produced from result and 3 original control testing
   -each of the 1.5 ml tubes are labeled to match original vial from which they came





7. Now nine 1.5ml tubes are then spun down for 10 minutes at 41,000 rpm.
8. All 9 tubes must be mixed with 1x buffer made from 2x buffer (950ul) and βME (50ul) solution
   -prepares for SDS page
9. Begin preparing for the SDS-PAGE by creating the Separating gel and the Stacking gel for standard SDS
10. Once the gels are set and ready, load the each of the 1.5ml sample tubes as well as the ladder into the wells accordingly
11. Once the SDS-Page is complete, then stain with coomassie Blue
12. column purification
    -running liquid solution through Avidin column.
    -biotin tag on protein then attaches to avidin column allowing for separation from waste.
    -then washed with excess biotin to break avidin-biotin bond with column.
    -protein is then forced through the column to a 1.5ml tube to retrieve concentrated protein
13. test protein for a peak 280 nanometers absorption
14. run SDS-page for conformation
15. Celebrate or cry.... Probably cry but that's ok. Let it out. We gotta try again.

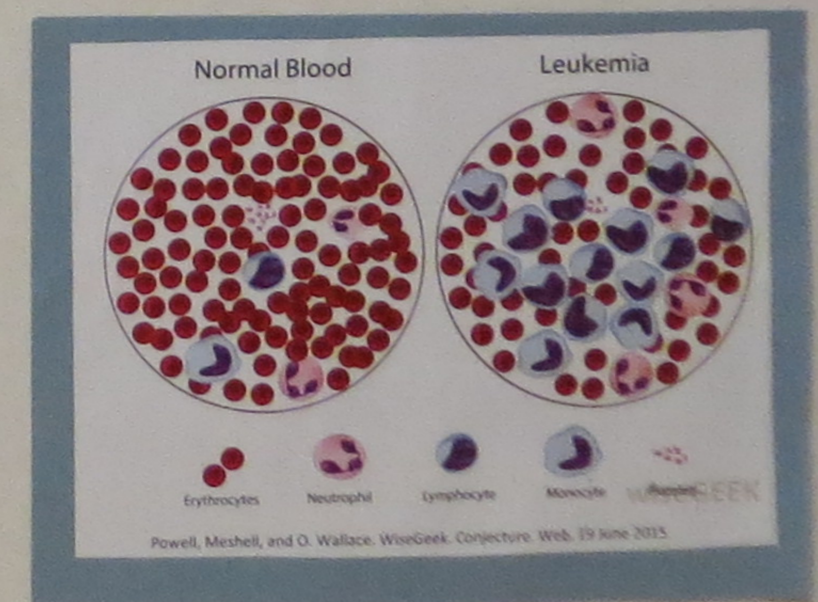
Future Directions

In the future, the project might take the direction of perhaps looking into what point the induction should happen in order to produce the highest results. This is from the likely event that the bacteria may not induce.

For the future, a new project might be to test whether or not the antagonist 8-83 truly does block signals from CCL19 to CCR7. This can be done with a simple gel experiment to see if in the presence of 8-83, CCR19 stays away from CCR7, and by removing 8-83, it should in turn migrate towards it.

Then there's the task of making the protein into a medication. To see which method is best suited for delivering the 8-83 to the desired site.

There could also be an experiment to see how long the side effects of the medication last as well as how severe they become.



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