Comparison of Protein Expression in the Cytoplasm vs Periplasm in E. coli

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BACKGROUND INFORMATION

T-cell acute lymphoblastic leukemia (T-ALL) is a type of cancer that is characterized by an over production of white blood cells, mainly in the bone marrow. This year, there is an estimate of 6,950 diagnoses, 60% of the cases will be children under the age of 21. If not treated, T-ALL has the potential to rapidly spread from the blood stream to several organs in the body including the brain where T-ALL can hide from treatment.

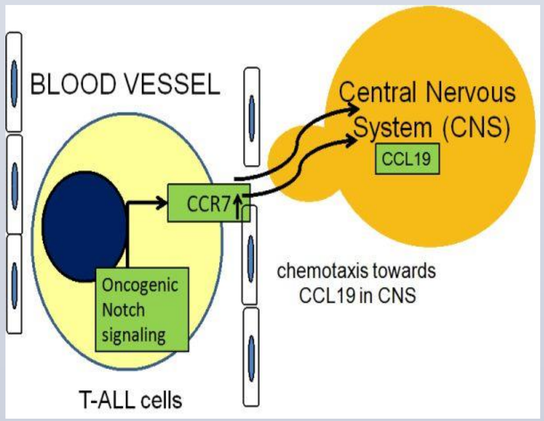
Current treatments for T-ALL are chemotherapy and radiotherapy that can reduce the amount of cancerous cells however it can also kill healthy cells. Although these treatments might be effective, there is a chance for leukemia relapse. Thus there is a need to develop more effective and less aggressive treatments.

It is well known that T-ALL cells use C-C Chemokine Receptor 7 (CCR7) to get into the brain (Buonamici & Lanis, 2009) via signaling of the C-C chemokine ligand 19 (CCL19) from the brain. It is needed to stop the migration of T-ALL into the brain so that leukemic cells can be more easily killed in the blood stream.

HYPOTHESIS

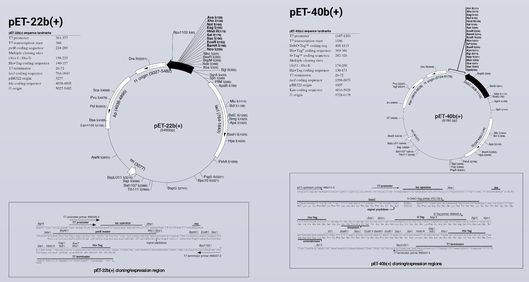
Dr. Vines' groups had problems making and purifying this antagonist in bacteria from the periplasmic space of E. Coli.

We hypothesize that by expressing the antagonist 8-83 in the cytoplasm of E. Coli (in the Shuffle NEB strain that allows disulfide bond formation in the cytoplasm) and not the periplasm, protein expression and folding might be more effective.



MATERIALS & METHODS

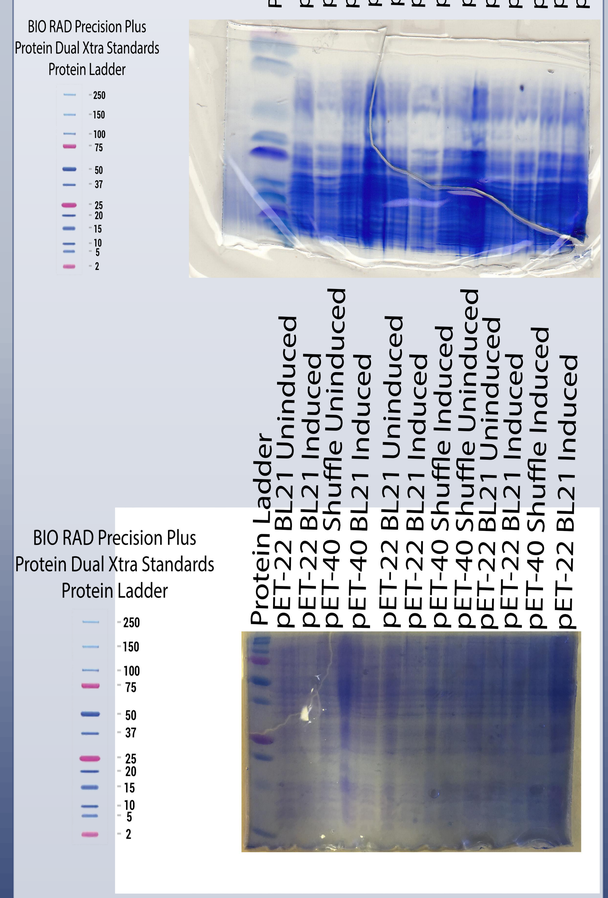
We will transform(insert DNA into bacteria) the vectors (circular DNA) pET-40 and pET-22 that contain the 8-83 DNA insert into the DH5α and NEB Shuffle Turbo *E. coli* strains. pET-40 was selected because it has a pelB sequence that will promote periplasmic localization of 8-83, while pET-22 has a cytoplasmic localization signal to induce expression of a soluble protein in the cytoplasm. Then we will grow cultures overnight from the colonies that we got from transforming and purify the bacteria by mini prepping them. A series of buffers will be used to break open the cells and to purify DNA. We will use the Nano drop to confirm the purity of the DNA. We will run a get the gel diagnostics which is the process where we run an agarose gel to examine whether the plasmid has the size expected. That is, the plasmid should have 5.5 kilo base pairs that corresponds to the vectors plus 8-83. We will transform the pET-22 and pET-40 into BL21(DE3) and Shuffle. We will grow the cells overnight in a shaking incubator at 37°C and 250 rpm. We will measure the optical density of E. Coli cultures to determine growth characteristics and the log stage of growth. Optical density is a method for calculating the concentration of E. Coli and their rate of growth. We will induce 8-83 expression at the log (exponential) stage of growth. Induction will be achieved exposing E.coli to isopropyl β-D-1 thiogalactopyranoside (IPTG) for 4 hours so that transcription of 8-83 can occur. We will run our Sodium Dodecyl Sulfate Polyacramdie gel electrophoresis –(SDS-PAGE) to examine whether the 8-83 was produced by E. Coli. We will purify which will get proteins 8-83 in a soluble and functional form by using the His-tag within the vectors because the string of histidine residues bind to several types of immobilized metal ions including nickel etc., under specific buffer conditions. Materials: Agarose gel (checking size of DNA) Sodium dodecyl sulfate, poly acrylamide gel electrophoresis (SDS-PAGE), analysis equipment, Gel dock XR imaging camera, broth media, 4x Tris CI/SDS pH 6.8, 1x Tris/Glycine/SDS spectrophotometer, Centrifuge, Incubator, the shakers around lab, isopropyl β-D-1-thigalactoyranoside, BL21, Shuffle, and D5aplha E. coli bacterial strains.



OBJECTIVE

Dr. Charlotte Vines' lab is working on a CCR7 antagonist originally described by Pilkington, to block the CCR7 directed migration of T-ALL into the brain. This antagonist, 8-83, is generated by cutting off the first seven amino acids of CCL19. The goal of this study is to develop a CCL19 antagonist and thus prevent leukemia cells from entering the brain.

RESULTS



DISCUSSION & FUTURE DIRECTIONS

Once we get the protein we will purify and use a migration assay to examine if 8-83 binds to CCR7 and stops migration of T-cells towards CCL19.

Discussion: after this we will purify the DNA then we will use the nickel spin columns which will the his-tag will stick and will cause all other proteins to wash off all the things you won't need so that you are left with the his-tag.

CONCLUSION

It's inconclusive because you could not tell the difference between the uninduced samples and the induced samples for pET-40 and pET-22. For pET-22 and pET-40 in the all of lanes it shows induction for the induced and the uninduced.

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ACKNOWLEDGEMENTS

We would like to thank Work with a Scientist Program, EPISD, Dr. Vines, as well as Research Assistants Zachary Parada and Gisel Flores, and family/friends for supporting us.

This project is sponsored by National Science Foundation, No. DRL---1322600