Using Inclusion Bodies to More Efficiently Isolate CCL19 Antagonist 8-83

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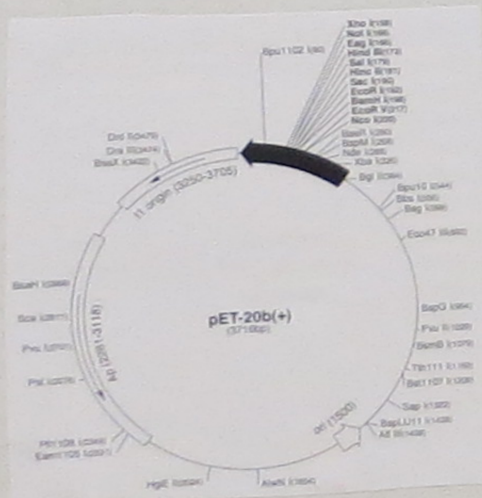
Background

To be able to study the effects of the antagonist 8-83, this protein must first be at its most pure state. 8-83 will be used to block binding of the normal ligands- chemokine ligands 19 (CCL19). This would inhibit the migration of the T-Cell Acute Lymphoblastic Leukemia (T-ALL) cells that use chemokine receptor 7 (CCR7) to direct where the T-ALL cells migrate. To be able to use 8-83, this antagonist must first be cloned into PET vector 20b(+), a vector that has the properties to send 8-83 into the periplasmic space of a bacteria cell membrane. Placing this vector in the periplasmic space will allow us to obtain a more purified protein.

Objective

Our main objective is to purify the protein 8-83 by sub-cloning it into PET vector 20, a vector that contains the properties required to transport 8-83 into the periplasmic space of bacterial cells. Once inside of the periplasmic space, this protein will form inclusion bodies, which are insoluble aggregates of highly purified 8-83.



Hypothesis

By expressing 8-83 into the periplasmic space we suspect that we will get a better protein expression.

If we retrieve a purified protein, like we hypothesize, 8-83 can be used as an antagonist to inhibit the migration of T-ALL into the Central Nervous System (CNS), where it can hide behind the blood brain barrier. Once isolated, 8-83 can blind the CCR7 expressing T-ALL cells so that they can no longer hide behind the blood brain barrier, but instead remain in the blood stream where they can be destroyed with chemotherapy.

Methods and Materials

The way that we will get the 8-83 pure protein expressed is through using a DNA vector. In the DNA vector, the RNA will be transcribed from the DNA vector and translated into protein.

Initially, to begin our experiment, we will transform the DH5α bacterial cell line with PET Vector 20b(+).

We then will grow it for 30minutes-1 hour at 37 degrees Celsius. This gives the cells ample time to express the antibiotic resistance enzymes and reach log phase of growth.

When the cells are in log phase of growth, we will plate it on ampicillin plates. At the same time, we will streak our induction control.

After our induction control is streaked, we will make sure it grew to know for a fact that the PET-20 vector is being selected.

We then will plate our bacteria on ampicillin because the vector that we chose to sub clone 8-83 in, PET Vector 20b(+), is ampicillin resistant, therefore many colonies of bacteria with the PET- 20 vector will grow and be efficient to use.

Once we have chosen a colony we will place it in lysogeny broth (LB), a media that contains the bacterial food to let the bacteria grow.

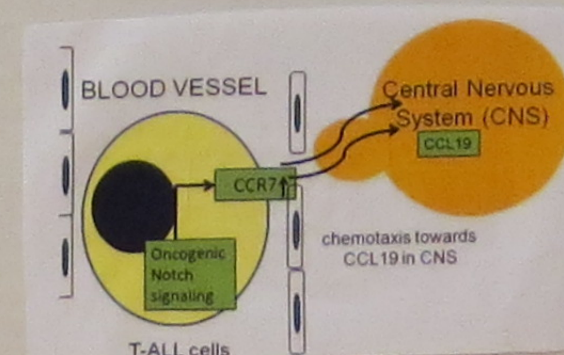
After placing the bacteria in the media, we will place it in an incubator/shaker, which shakes the tube containing the bacteria allowing it to grow over a period of 12-16 hours.

At this point, we will measure the optical density by using a machine that transmits a light and measures how dense our bacteria culture is, the machine is a Photo Spectrometer. The ideal measurement of optical density for our bacterial cultures ranges from .6-.8 nanometers because between this range, the cells are in their optimal phase of their growth.

We will follow a protocol called miniprep, by using a miniprep kit that is provided. This protocol contained a varied number of buffers; P1, P2, PB, and PE all used to extract pET-20 vector from DH5α and the bacterial we used to grow the vector in. Once the supernatants have been retrieved and centrifuged, we may extract the purified PET-20 vector. Once purified, the vector may be used to encode the fusion protein 8-83, which directs it into the periplasmic space of a bacterial cell. In this periplasmic space, a protein is being expressed.

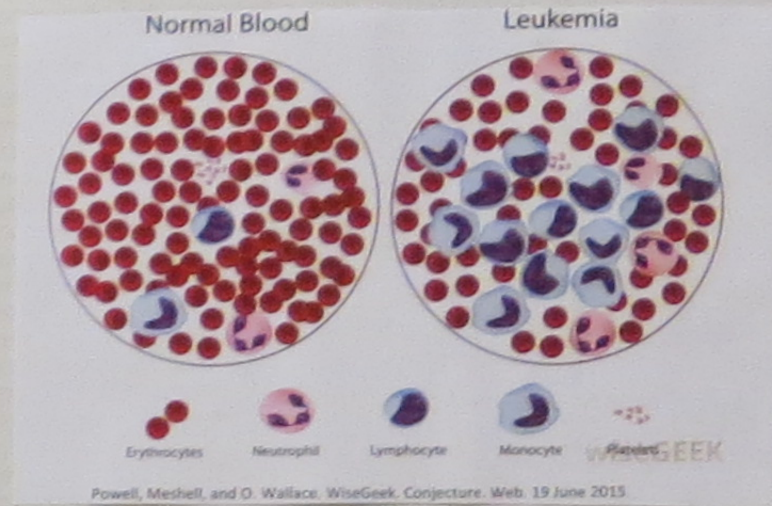
Once expressed, the protein may be removed and separated from the bacteria by a method called freeze/ thaw. This process involves slowly freezing the cell, doing so will create ice crystals that will pierce through the membrane setting the pure, expressed protein free. If the protein is successfully released, it will begin a whole new project that can change the future of Leukemia patients.

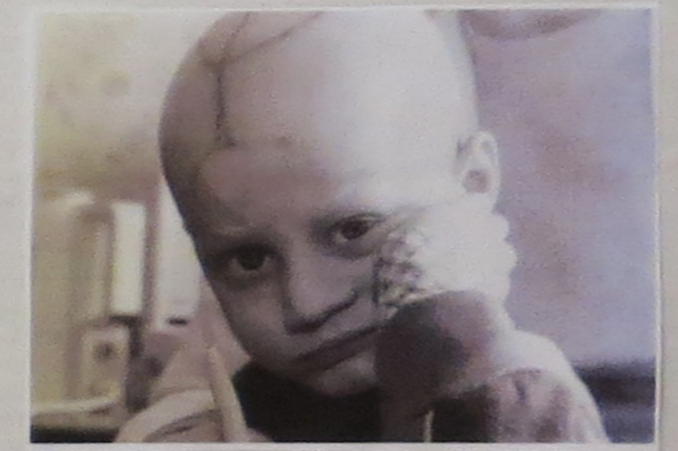
The timeline for our research lasts for six weeks. By the end of a six week period, we should have our purified 8-83 protein and have tested out our second project in correlation to our initial success.



Pediatric T-Cell Acute Lymphoblastic Leukemia

Pediatric T-cell acute lymphoblastic leukemia (T-ALL) is a type of cancer that occurs in ones body as a result of having too many white blood cells. Because it is acute, it progresses quickly and if not treated soon enough, the results can quickly turn fatal. Leukemia cells invade the blood quickly and can spread into the lymph nodes, liver, and central nervous system. This specific type of cancer affects children ages 21 and below, however the greater percentages of children that are diagnosed with this disease are between the ages of 8 and 9 years old.





Future Directions

Once we have obtained the most purest form of 8-83, we can use this protein to inhibit the migration of T-ALL cells to the central nervous system (CNS). In order to do this, we will place the 8-83 into a migration assay to observe if 8-83 prevents CCL19 from activating CCR7 to transport the T-ALL cells to the CNS.

Our ultimate goal is to prevent T-ALL cells from passing the blood brain barrier. Our reason for doing so is to inhibit the migration of T-ALL cells into the CNS in order to prevent the use of more invasive and detrimental treatments to patients.

If our migration assay is successful, we can begin testing this migration inhibiter on laboratory rats for further investigation.

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