Impact of E.coli Strain Variation and Comparison in Protein Expression and Purification of the CCL19 Antagonist 8-83

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Introduction

T-Cell acute lymphoblastic leukemia (T-ALL) is mainly characterized by the over production of white blood cells. It begins in the bone marrow, spreads to various organs, and may travel to the brain and spinal fluid. [9] This is especially dangerous once it has crossed the blood brain barrier. T-ALL most commonly affects children and young adolescents.

Treatment for T-ALL mainly consists of intravenous and intrathecal chemotherapy. Patients typically go into remission not long after commencement. However, leukemia may recur, in some cases called extramedullary relapse, which is a recurrence of T-cells in a specific part of the body. [1] This usually calls for more intense treatment, such as cranial radiation therapy, which can be especially dangerous.

Long-term effects and risks of chemotherapy and radiation include: heightened risk for future cancer, respiratory problems, stunted growth, learning disabilities, fertility issues, brain damage, bone damage or osteoporosis, loss of endocrine function and even mental/emotional or psychological issues. [2]

Despite treatment, patients may still be in danger of having remaining T-cells traveling to the brain, out of reach of radiation therapy. T-cells receive signals from a protein produced in the brain: CCL19 (chemokine ligand type 19). The signal causes them to produce a protein of their own called CCR7 (chemokine receptor type 7). This allows them to follow CCL19 beyond the blood brain barrier, making it near impossible to eradicate the cells from that point due to the delicate area. Radiation therapy is likely out of the question due to the high damage risks.

To solve this, we will use an antagonist called 8-83, and have them attract the CCR7, destroying the opportunity of CCL19 being able to attach to CCR7. This will keep the T-cells in the blood stream, ensuring that they will not pass the blood brain barrier, and it will be easier to destroy the T-cells. However, the proteins that are created become toxic to the cell, killing them.

8-83 proteins have been typically expressed in our laboratory in a bacterial strain called BL21, but the proteins soon become toxic to the bacteria. Based on the codons present in 8-83, we have found that the Rosetta strain expresses matching codons that are rare in E.coli. We have two strains: BL21 Codon Plus (DE3)-RP and Rosetta (DE3) as variants that may yield new results.[8]

Objective

Our goal is to induce protein production using different bacterial strains to determine if the host strain plays a significant role in facilitating the production of 8-83.

Hypothesis

By using a different E.coli strain that expresses the same rare E.coli codons as 8-83, we will successfully express the protein without the cells dying. This will block migration of the T-cells into the brain, keeping them in the bloodstream instead. The BL21 Codon Plus (DE3)-RP will be our control, and Rosetta (DE3) will be our variant.

Procedures

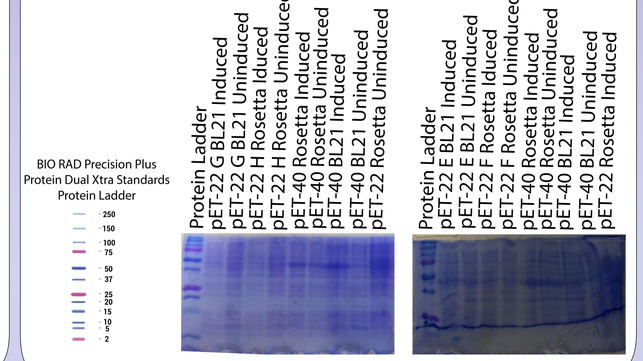
1. Transform pET-40 with 8-83 insert into E. coli.
2. Grow bacteria by plating them on media and antibiotics.
3. Incubate bacteria at 37 degrees Celsius and shake at 250 rotations per minute overnight.
4. Purify DNA, using mini prep.
5. Nano drop to examine DNA purity.
6. Examine DNA size with an agarose gel.
7. Examine the optical density (OD) of the cells and induce at 0.8 OD with ITPG (it will remove the oppressor from the lac operon)
9. Run SDS-PAGE to examine whether the protein is expressed.
10. After protein expression, purify the protein until it is in a functional form.

Equipment

- Spectrophotometer
- Pet-40 Vector
- Pet-22 Vector
- Rosetta (DE3) Novagen
- BL21 (DE3) codon plus-RP

Results

Our SDS-PAGE Gel has shown protein expression of 8-83 in both bacterial strains: BL21 and Rosetta, and with both vectors: pET-40 and pET-22. When we look at the pET-40 results, we see that not only did BL21 have a stronger expression of 8-83 compared to Rosetta, it also did not express 8-83 in the uninduced conditions where Rosetta did. With regard to pET-22, BL21 and Rosetta both had some protein expression which was stronger for the uninduced samples as compared to the induced samples.



Conclusions

It was hypothesized that the Rosetta strain would be more effective than BL21 at expressing 8-83. However, our results proved contrary to our predictions, and BL21 was more effective at expressing 8-83. Additionally, its expression was more tightly regulated when using the pET-40 vector, whereas using the pET-22 vector none of the bacterial strains expressed the protein under the induced condition but they did under the uninduced condition. Perhaps expression of 8-83 with pET-22 in either strain is toxic. To conclude: using pET-40 in BL21 to express 8-83 may be more effective than using pET-22 and/or the Rosetta strain.

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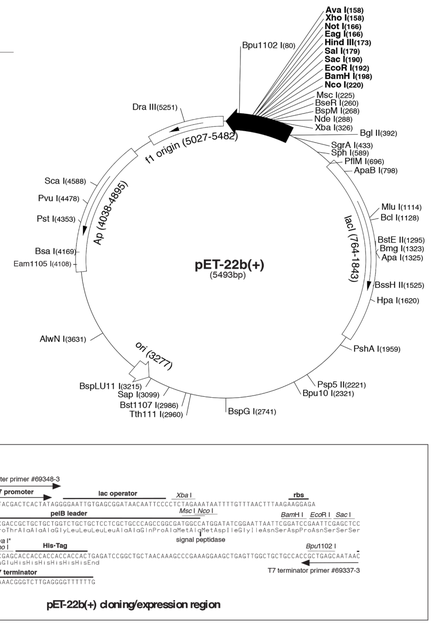
Acknowledgments

This project is sponsored by National Science Foundation, No. DRL-1322600.
We'd also like to to thank the staff of the biomedical research center (BBRC) for the services and facilities provided, and give a special thanks to Zach Parada, Gisel Flores, and Dr. Charlotte Vines for their constant support and guidance. We appreciate the time and effort that was put into preparing our presentations. It has been a great opportunity to learn and get involved with this program.

pET-22b(+)

pET-22b(+) sequence landmarks	
T7 promoter	361-377
T7 transcription start	360
pelB coding sequence	224-289
Multiple cloning sites	
(*Nco* I - *Xho* I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	764-1843
pBR322 origin	3277
bla coding sequence	4038-4895
f1 origin	5027-5482



pET-40b(+)

pET-40b(+) sequence landmarks	
T7 promoter	1187-1203
T7 transcription start	1186
DsbC•Tag™ coding seq.	408-1115
His•Tag® coding sequence	369-386
S•Tag™ coding sequence	282-326
Multiple cloning sites	
(*Syr*I - *Xho* I)	174-250
His•Tag coding sequence	150-173
T7 terminator	26-72
lacI coding sequence	1594-2673
pBR322 origin	4107
Kan coding sequence	4816-5628
f1 origin	5724-6178