Triggering Chemokine (C-C Motif) Receptor 7 Mediated Apoptosis

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Background

- Leukemia affects over 3,000 kids a year in the United States. The current treatment is chemotherapy and radiation.
- However, these treatments have secondary effects on patients such as less energy, may bruise or bleed easily, loss of hair, nausea, vomiting, mouth sores, lower resistance to infection, and in some cases infertility.

- Some patients that have been "treated" have a high probability of relapse and after this there is only one way to end this: Death.
- Our experiment is to trigger apoptosis to cure Leukemia by ONLY killing T-cells, therefore the patient's health won't be at risk.

What is Apoptosis?

Apoptosis: A Programmed cell death or a " Cell Suicide". It's a natural process that eliminates damaged, unwanted or dangerous cells from the body.

- In multicellular organisms, cells that are no longer needed or are a threat to the organism are destroyed by apoptosis.

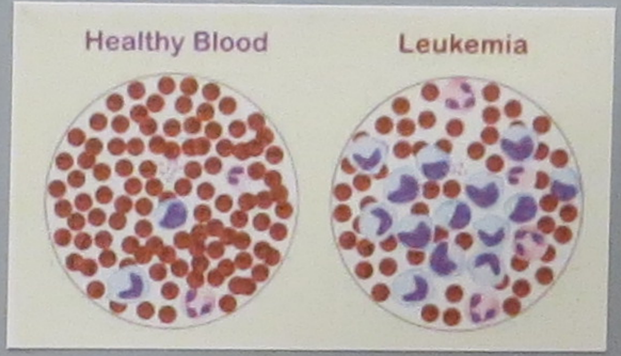
How is Apoptosis Trigger?

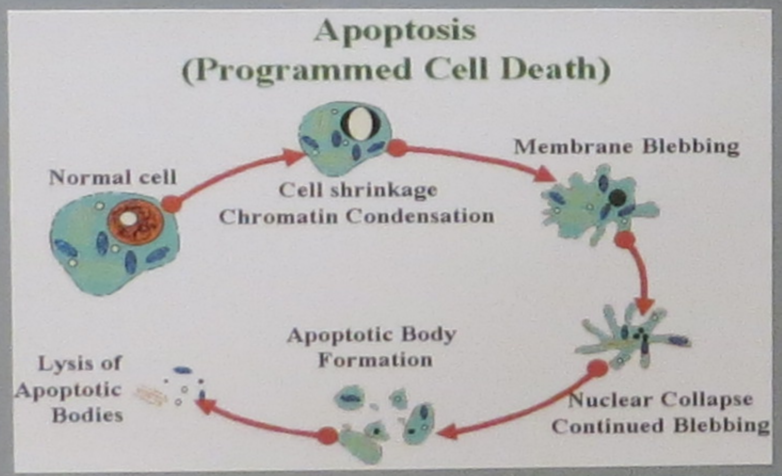
We hope to trigger apoptosis by Expressing truncated Chemokine (C-C Motif) ligand 21, CCL21 known as mSLC4, with pinpoint Xa. Then purify it with biotin(to cut the Biotin and mSLC4 we will need Factor Xa), hoping to trigger Apoptosis Assay Annex V.

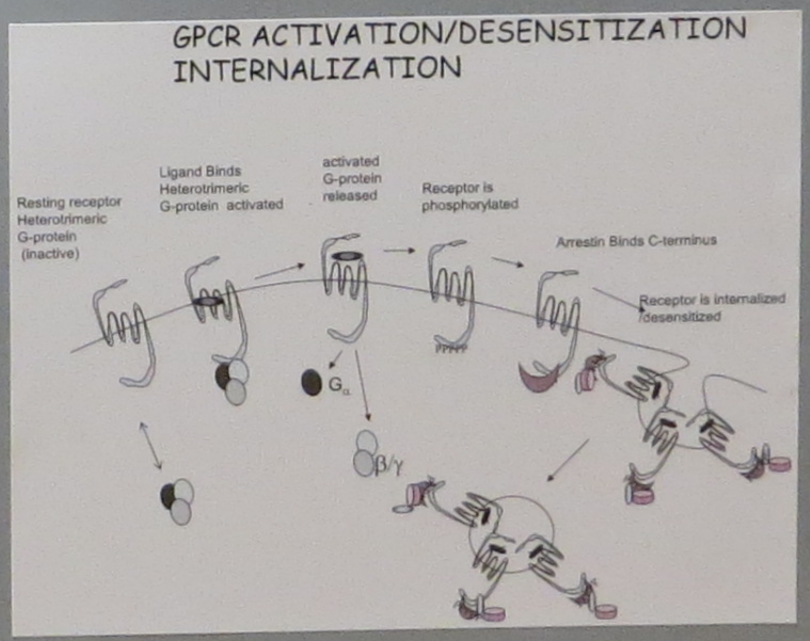
Note: The more mSLC4, the more dead T-cells we hope to have.

Hypothesis

We are using (truncated CCL21) mSLC4 because Dr. Vines did a paper on apoptosis in 2004, stating that if during the GPCR activation the arrestins will fail to bind to the receptor and the cell will undergo apoptosis. When we add mSLC4 to the cell receptor to bind CCR7 on the surface of the cell. The mSLC4 does not cause CCR7 to become phosphorylated, since the CCR7 won't be phosphorylated, Arrestin will not bind







Materials

Ice, mSLC4 - PinPoint™ Xa, PinPoint™ Xa Control Vector, DH5 Alpha, BL21, 3ml SoftLink™ Soft Release Avidin Resin, 1 PinPoint™ Purification Column, 1ml Biotin, 100mM (pH 7.2), 1 Protocol, ampicillin stock solution, IPTG, 100mM, LB medium, lysozyme (Sigma Grade VI, Cat.# L2879), sodium deoxycholate (DOC), Triton® X-100, acetic acid, 10%, cell lysis buffer, ethanol, 20%, phosphate buffer, 100mM (pH 7.0), Factor Xa Protease (Cat.# V5581), Factor Xa 10X reaction buffer, sample 1X buffer, sodium deoxycholate (DOC), trichloroacetic acid (TCA), Tris-HCl, and 20mM (pH 8.0)

Methods

1. Grow Bacteria in both BL21 and DH5 Alfa
2. Make Luria Broth Media
3. Make Small-Scale Culture and Induction
4. Preparation and Regeneration of SoftLink™ Resin
5. Column Capture
6. Cleaving the Fusion Protein

Measuring Process

The externalization of phosphatidylserine (PS) is one of the leading indicators of apoptosis. In normal viable cells, PS is located on the cytoplasmic surface of the cell membrane. However, in the intermediate stages of apoptosis, PS is translocated from the inner to the outer leaflet of the membrane, exposing PS to the external cellular environment where it can be detected. Highly fluorescent annexin V conjugates provide quick and reliable detection methods for studying the externalization of phosphatidylserine.

So, we will measure apoptosis by AnnexinV, which binds two certain membrane lipids that are exposed during apoptosis

If our process works we will inject purified mSLC4 into a mouse model of human T-ALL through an intravenous injection, which we'll preform through the major veins in the tail.

By doing our process we will target all the T-ALL cells, this may cause a low immune system but cure leukemia.

References

http://www.ncbi.nlm.nih.gov/books/NBK26873/
http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCL2
https://scholar.google.com/citations?user=...
http://www.cancercompass.com/leukemia-information/side-effects.htm
http://wolfson.huji.ac.il/purification/PDF/Tag_Protein_Purification/Biotinylated/PROMEGA_PinPointXa.pdf