



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

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ABSTRACT

Leukemia affects over 3,000 children each year in the United States. The current treatment is chemotherapy and radiation. However, these treatments have secondary effects on patients such as loss of energy, bruising or bleeding, loss of hair, nausea, vomiting, mouth sores, lowered resistance to infection, and in some cases infertility. Patients that have been treated have a high probability of relapse and after this the child's odds for survival plummet. Our goal is to trigger apoptosis to cure Leukemia by ONLY killing T-cells by the process of apoptosis, therefore the patient's health won't be at risk.

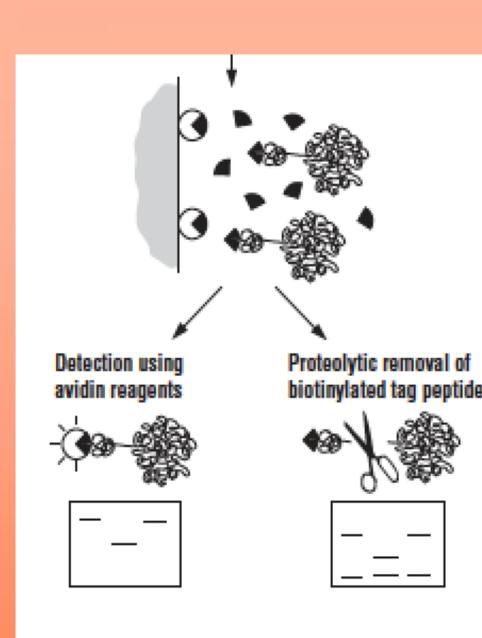
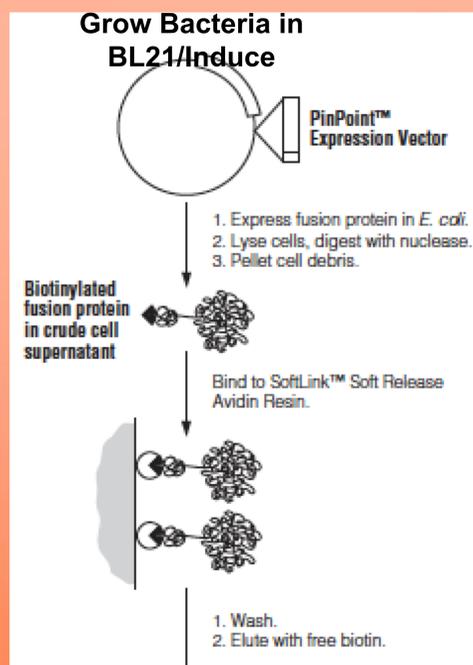
Apoptosis is a programmed cell death or a "Cell Suicide". It's a natural process that eliminates damaged, unwanted or dangerous cells from the body. We hope to trigger apoptosis by exposing cells to mSLC4.

BACKGROUND

We propose to trigger apoptosis by exposing acute leukemic T cells to truncated form of (C-C Motif) chemokine ligand 21 (CCL21) known as mSLC4. To this end we will express mSLC4 using the Pinpoint Xa system which fuses a biotinylation tag to the N-terminus of mSLC4. We will purify it on a monomeric avidin column, and elute mSLC4 with biotin. To remove the biotinylation tag from the mSLC4 we will use Factor Xa. To determine if there is apoptosis, we will assay using Annex V.

Our work is based on an observation made by our lab in 2004, where we found that during GPCR activation, if the arrestins fails to bind to the receptor, the cell will undergo apoptosis. Normally full length CCL21 causes the receptor to activate for a short amount of time, without recruiting arrestin. When we add mSLC4 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. We hypothesize that this will induce Apoptosis. The externalization of phosphatidylserine (PS) is one of the leading indicators of apoptosis. In normal viable cells, PS is located on the cytoplasmic side 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 by flow cytometry.

MATERIALS & METHODS



RESULTS



Unfortunately, after six weeks of working in the lab, we were not able to trigger apoptosis. After so many tries, all we got was a low level of induction. This is because Pinpoint Xa is a low copy vector and therefore, it is very hard to induce. We tried to purify the protein that we express, but did not succeed. We think that this is because our sodium levels are very low, which is not clearing up all the debris and as a result, mSLC4 could not be purified.

DISCUSSION/FUTURE DIRECTIONS

At present we are trying to purify mSLC4 with the Monomeric Avidin column. However, we are also trying to get better expression of our protein, since we had low levels of induction. Ultimately, our goal is to stop CCR7 from becoming phosphorylated and therefore triggering apoptosis.

As for future directions, we want to figure out how to get better induction of mSLC4. We would also want to purify our protein and start testing it on mice and hopefully it will move quickly into clinical trials.

REFERENCES

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