

Investigating the Role of the C-Terminal Domain in the Cxcr3 Receptor in Migration of Breast Cancer Cells

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[Project Proposal Revised DRAFT]

**I. Statement of Purpose**

Cxcr3 is a receptor with an n-terminal end that sits outside of the cell and a c-terminal end that reaches into the cell (cytoplasmic portion). The protein Cxcl10 binds to Cxcr3 and has been shown to induce cancer cell migration (related to tumor metastasis). Please refer to the “III. Prior Research” section of this proposal for an in-depth introduction to my project.

The main question I plan to research is “What is the main function of the c-terminal end of Cxcr3 in breast cancer cell migration?” My hypothesis is that this cytoplasmic portion of Cxcr3 is necessary for Cxcl10-induced cell migration. The goal of my project is to investigate a mutant gene encoding for the shortened chemokine receptor Cxcr3. Another question that this project may address is if treatment options targeting this c-terminal end of Cxcr3 are possible. My current project is planned to take the cell line expressing my mutant gene from my previous internship and comparing it to normal breast cancer cells for cell migration.

**II. Background**

I spent 8 weeks as a summer intern at the Translational Genomics Research Institute, working in the breast cancer research lab. My research project was a part of the larger investigation of the role of chemokine signaling in breast cancer. The lab was investigating chemokine Cxcl10 signaling that induces cell migration and tumor metastasis in breast cancer. My project was to determine the function of the c-terminal domain (end part of the protein) of Cxcr3, the receptor for Cxcl10, in breast cancer cell migration. The hypothesis was that the cytoplasmic portion (inside the cell) of Cxcr3 was important in interacting with downstream components for cell migratory signaling. My goal was to create a Cxcr3 mutant with a c-terminal deletion. I designed primers for a PCR process to clone the mutant gene. To design these primers, I found the gene sequence in the NCBI database and wrote out the complementary bases of 23 nucleotides. I cloned the PCR’ed mutant DNA into a retroviral vector and put the construct in breast cancer cells in culture. I grew and selected for the cells expressing my mutant gene towards the end of my internship.

**III. Prior Research**

Triple-negative breast cancer (TNBC) lacks common breast cancer receptors (estrogen, progesterone, and HER2) that can be targeted by therapeutics. With current treatments, over 40% of women with TNBC see recurrence within 5 years, and less than 12% with distant metastatic TNBC survive past 5 years (American Cancer Society, 2023).

Tumor suppressor genes encode for proteins that inhibit tumor formation and development. Up to 34% of all breast cancer tumors are deficient in the tumor suppressor Inhibitor of Growth 4 (ING4) and correlated with poor patient survival. Chemokines are signaling proteins that induce directional movement mostly in immune cells. Higher concentration of Cxcl10 in ING4-deficient TNBC incites cell migration and tumor metastasis in experimental systems (Tsutsumi et al., 2024). Clinically relevant, patients with Cxcl10-high/ING4-low expressing breast tumors have significantly reduced disease-free survival (Tsutsumi et al., 2022).

Cxcl10 binds the seven transmembrane domain G protein-coupled receptor Cxcr3 to signal cell migration. The carboxyl-terminus is the end of an amino acid chain. The carboxyl-terminal intracellular domain of Cxcr3 has been shown to promote chemotactic migration in lymphocytes (immune system response cells) (Colvin et al., 2004). However, the specific effects of Cxcr3’s carboxyl-terminus in TNBC is unknown.

**IV. Significance**

This project’s goal is to investigate the specific effects of Cxcr3’s carboxyl-terminus in TNBC. To start this project, we create a Cxcr3 protein missing 40 amino acids on the c-terminus and deliver it into a TNBC cell line. The project hypothesis is that “the carboxyl-terminal domain of Cxcr3 is required for Cxcl10 induced ING4-deficient breast cancer cell migration.” In addition, the experimental results may also address questions about if the third intracellular loop of Cxcr3 can incite cell migration without the carboxyl-terminus.

This project will provide insight into the function of the C-terminal domain of Cxcr3 in TNBC metastasis, which could have implications in future treatment options for TNBC. These possible future treatment options would improve TNBC patient outcomes. My findings will be used in the research on chemokine-induced ING4-deficient breast cancer cell migration. Lastly, I believe my final presentation might shed light on the importance of signaling molecules in TNBC, seeing as there are currently limited treatment options for the disease.

**V. Methodology**

To investigate the C-terminal domain of Cxcr3, I will mainly be working in a breast cancer research lab with my mentor Dr. Suwon Kim. I will start my project by consolidating the previous work I have done on a Cxcr3 mutant and looking into other research articles on the chemokine receptor. In the lab, I plan to start by troubleshooting the unsuccessful western blot procedure from the end of my previous work on the project. I will examine the restriction enzyme digest performed on the retroviral vector to confirm that the vector insertion was successful. If the previous chosen vector with insert was incorrect, then I will identify a new vector to use and transfect a new breast cancer cell line. From there, I will conduct neomycin selection on the cell line and prepare the new batch of cells for western blot. The end goal of my work in the lab is to compare the cell line expressing my mutant gene to normal breast cancer cells for cell migration. My work outside the lab involves reviewing the likely mechanism of Cxcl10 binding. In the lab, the next step I will learn is performing a migration assay to compare the cell line expressing my mutant gene to normal breast cancer cells for cell migration.

**VI. Problems**

The main problems that I might face are detecting the mutant Cxcr3 protein on western blot and performing the necessary experiments in the project time frame (February to end of April). For example, migration assays on cells expressing my mutant gene might need more time after the two and half months. If I am unable to collect enough data in that time, I may have to pivot my experiment to focusing on examining the results I already collected and presenting the information I do gather on the c-terminal domain of Cxcr3. In addition, I might be able to diagnose the problem with my previous project’s western blot from examining the restriction enzyme digest and pivot my project towards exploring the possible solutions that could be used to further investigate chemokine-induced breast cancer cell migration.

**VII. Bibliography**

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