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The CXCR1 Axis: A Putative Therapeutic Cancer Stem Cell-Like Marker in Pancreatic Ductal Adenocarcinoma

Elizabeth Thomas
University of Nebraska Medical Center

Caitlin Molczyk
University of Nebraska Medical Center

Paran Goel
University of Nebraska Medical Center

Marie Powers
University of Nebraska Medical Center

Lubaba A. Zaman
University of Nebraska Medical Center

See next page for additional authors

The CXCR1 Axis: A putative therapeutic cancer stem cell-like marker in pancreatic ductal adenocarcinoma

Elizabeth Thomas, Caitlin Molczyk, Paran Goel, Marie Powers, Lubaba Zaman, Evie Ehrhorn, Samuel Cohen, and Rakesh K. Singh
Department of Pathology & Microbiology, University of Nebraska Medical Center, Omaha, NE 68198

Abstract

Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest survival rates of all cancers in the United States. Not only is PDAC found at the late stages, but patients also present with or develop chemotherapy resistance at an elevated frequency. Left with limited options for treatment, researchers are investigating new options for these patients. One major area of interest is the sub-population of cells in the tumor called cancer stem cells (CSCs). These cells are known for having high resistance to chemotherapy, along with propagating and re-building the tumor after most non-CSCs have been therapeutically targeted. Previous studies have determined CXCR4, ALDH1, CD24, CD44, and CD133 as markers for CSC-like PDAC cells. In the present study, we investigate the closely related CXCR1 as another possible marker and therapeutic target for PDAC CSCs. CXCR1 is known for its role in inflammation and wound healing. The CXCR1 axis includes the ligands CXCL6 and IL-8, both of which promote the progression of cancer. Previously, Ginesteir et al. has shown targeting the CXCR1 axis in triple negative breast cancer reduced CSC-like phenotypes *in vitro* and *in vivo*. Investigations of CXCR1 in PDAC demonstrate IL-8 induces increased tumorsphere formation *in vitro* (Chen et al.), leading us to investigating CXCR1 in PDAC CSCs. We hypothesize that PDAC cells with high CXCR1 activity also exhibit increased CSC-like characteristics and targeting CXCR1 will reduce those characteristics.

To investigate the role of CXCR1 in CSC-like phenotype of PDAC, we used the PDAC cell line CD18, along with its gemcitabine resistant (GemR) counterpart. We used the CXCR1/2 antagonist Navarixin at high enough concentrations to inhibit CXCR1. Using the previously found gemcitabine and navarixin IC50 concentrations for each parent cell line, we treated cells for 72 hours. Post-treatment, we analyzed the expression of several known CSC markers, CXCR1, and IL-8 through qRT-PCR and ELISA. We expected to see higher expression and activity of CXCR1 in cells with higher known CSC marker expression. We also anticipated that gemcitabine treatment would induce higher expression of CSC markers, whereas navarixin would exhibit lower expression. From our results, we see the beginning trends of gemcitabine treated cells having increased expression of the CSC markers and navarixin decreasing or not changing the expression levels. These results differ for IL-8, which undergoes an increase in expression when treated with both gemcitabine and navarixin, which may warrant further exploration into the role of ligands in CSC-like phenotypes. One possible explanation for this difference would be the regulation of IL-8 expression based on CXCR1 activity, as IL-8 interacts with CXCR1.

Introduction

PDAC is a highly deadly disease, with a 5-year survival rate of less than 10% of patients. One of the prominent causes of this low expectancy is resistance to a common treatment for PDAC, chemotherapy. One potential factor contributing to resistance is the presence of cells that exhibit cancer stem cell-like (CSC-like) properties within the tumor. These CSCs, like other stem cells, have the ability to self-renew and propagate, as well as differentiate into the variety of cells that make up the overall heterogeneous tumor (Figure 1A). CSCs also have the ability to enter and exit a quiescent state in which they aren't actively metabolizing high levels of proteins or dividing. Though the metabolic properties of these quiescent cells are limited, they express ALDH1 at a higher rate, which serves to convert toxic drugs into less toxic forms (Lee et al. 2018). Alongside ALDH1, CD24, CD44, CD133, and CXCR4 are published markers for cells with CSC-like properties. Another receptor suspected to play a role in CSC activity is the chemokine receptor CXCR1. In a normally functioning cell, the CXCR1 pathway promotes inflammatory responses and wound healing. In cancerous cells, where the pathway is not properly regulated, CXCR1 interacts with its ligands, CXCL6 and IL-8, to promote proliferation and metastasis of the tumor, as well as increase angiogenesis and chemotherapy resistance (Figure 1B). Previously published studies report targeting CXCR1 in triple negative breast cancer decreases the CSC-like cells both *in vitro* and *in vivo* (Ginesteir et al). In PDAC, Chen et al. treated tumor cells with exogenous IL-8 and found it has the capacity to induce tumorsphere formation *in vitro.*

Based on this background, it was hypothesized that targeting the CXCR1 pathway using the inhibitor drug Navarixin would negatively impact the CSC population within PDAC cells, as well as reducing the population of cells resistant to chemotherapy.

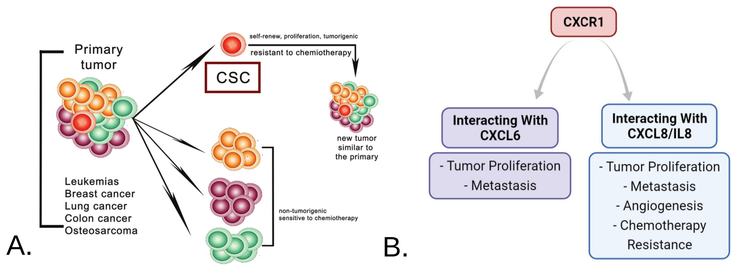

Figure 1. A. Cancer stem cell theory proposes that tumor cells originate and differentiate from a parent cancer stem cell. B. CXCR1 interacts with CXCL6 and IL-8 to promote various functions adverse to patient survival in cancerous tumors.

Experimental Strategy

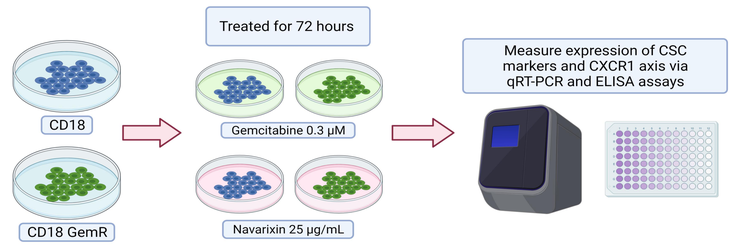

Figure 2. Outlines the generation of cell lines cultured with gemcitabine and navarixin for CXCR1 and CSC marker evaluation

CSC Marker Expression in Parent and GemR Lines

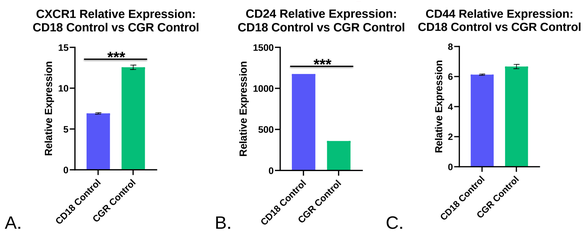

Figure 3. Expression of CXCR1, CD24, and CD44 in parent CD18 and derived CD18 GemR cell lines. A general increase in expression is observed from parent to GemR cell lines for both CXCR1 and CD44, while expression of CD24 decreases. *p<0.05 **p<0.01 ***p<0.001

Regulation of CSC Marker Expression by Gemcitabine

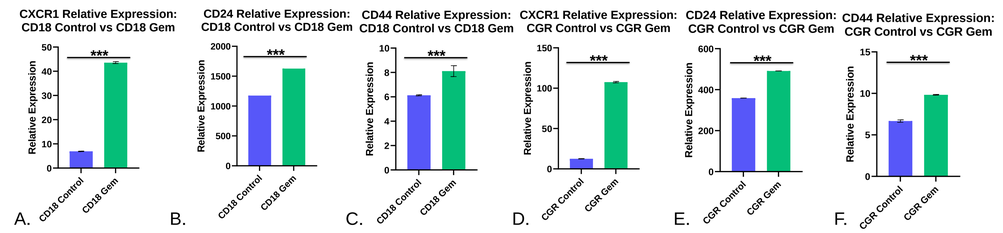

Figure 4. Expression of CXCR1 and CSC markers in parent and GemR cell lines when treated with gemcitabine for 72 hours. A significant increase in the expression of each marker is observed when the cells are treated with gemcitabine. *p<0.05 **p<0.01 ***p<0.001

CSC Marker Expression Inhibited by Navarixin

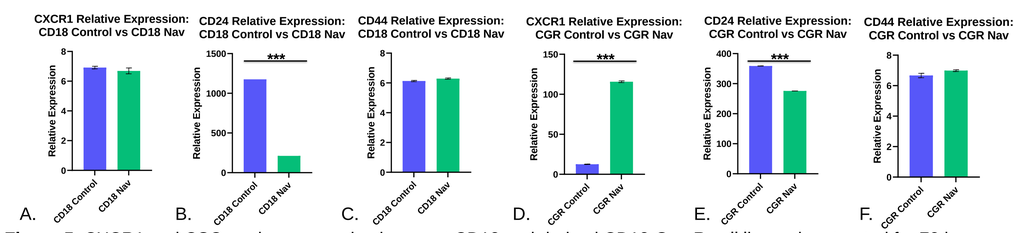

Figure 5. CXCR1 and CSC marker expression in parent CD18 and derived CD18 GemR cell lines when treated for 72 hours with Navarixin.CXCR1 expression experiences a general increase in the presence of navarixin, while CD24 expression decreases when treated with navarixin. CD44 does not have any definitive difference in expression when treated. *p<0.05 **p<0.01 ***p<0.001

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IL-8 Content in Parent and GemR Lines

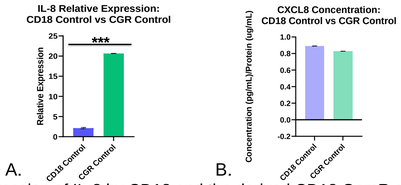

Figure 6. The expression of IL-8 by CD18 and the derived CD18 GemR cell lines. A. Relative expression of IL-8 from isolated mRNA. B. Protein expression of IL-8 as measured by ELISA, normalized to protein content. *p<0.05 **p<0.01 ***p<0.001

IL-8 Content Modulation by Gemcitabine and Navarixin

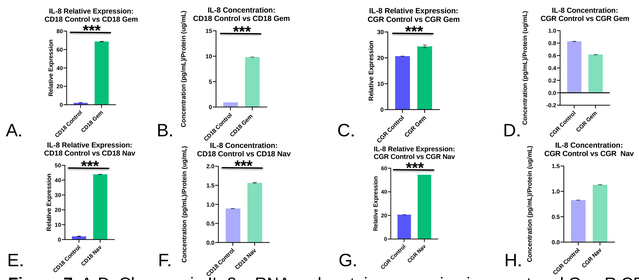

Figure 7. A-D. Changes in IL-8 mRNA and protein expression in parent and GemR CD18 cells when treated with gemcitabine for 72 hours. mRNA expression was measured via qRT-PCR, protein expression was measured by ELISA and normalized to protein content. E-H. mRNA and protein expression in cells treated with navarixin for 72 hours. An overall increase in both mRNA and protein expression is observed for both treatments. *p<0.05 **p<0.01 ***p<0.001

Preliminary Results

- Increased expression of CSC-like cell markers in GemR cell lines
- Increased expression of CSC-like cell markers in both parent and GemR cell lines when treated with gemcitabine
- Decreased expression of CSC-like cell markers when treated with navarixin
- Heightened CXCL8 mRNA and protein expression within parent and GemR CD18 cells when treated with gemcitabine or navarixin.

Future Direction

We will continue to explore the impacts of gemcitabine and navarixin on CSC-like cell markers through repetition of these experiments in order to compare results and formulate more definitive data. At this point in time, some of the results are not clear enough to draw a thorough conclusion. Once this is done, we will study the tumorsphere formation capabilities of untreated cells expressing high CXCR1 levels as another means of determining the CSC-like properties of CXCR1-high cells.

Moving forwards, it would also be beneficial to look into potential causes behind the occasional increases in CSC-like cell marker expression when treated with navarixin and decrease in CD24 expression in the GemR cell line as compared to the parent cell line.

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