Effects of cingulin and SPTBN1 protein knockout on pancreatic ductal adenocarcinoma tumor cell polarity and subtype

Sarah Tian, Mentors: Dr. Jen Jen Yeh and Sandy Zarmer

Abstract

This project aims to discover how knocking out the cell polarity proteins cingulin (CGN) and spectrin beta, non erythrocytic 1 (SPTBN1) affects PDAC tumor cell polarity and growth. Inducible exogenous CGN and SPTBN1 constructs will be transduced into PDAC organoids, and afterwards CGN and SPTBN1 will be knocked out with CRISPR. The tumor organoids will then be viewed with immunofluorescence microscopy to assess cell polarity and subjected to a CellTiter-Glo growth assay to assess cell viability. To study the effect of CGN and SPTBN1 on cell viability, a Bravo Pipetting protocol was developed to plate efficient organoid growth assays. The CGN exogenous construct was created and validated, SPTBN1 was sequence validated and initial immunofluorescence staining of CGN and SPTBN1 in a classical organoid line before knockout was negative. It is possible that the inability to stain for the proteins is due to non-specific primary antibodies, requiring optimization for staining organoids or a need for better control lines.

Introduction

- Pancreatic Ductal Adenocarcinoma (PDAC) comprises 90% of pancreatic cancers, has a 5-year survival rate of only 9% (American Cancer Society, 2020) and is the 4th leading cause of cancer deaths worldwide.¹
- PDAC has a classical and a basal subtype with different gene expression profiles. The basal subtype has significantly worse prognosis and response to current treatments.²
- Classical tumors have a more organized structure with defined ducts compared to basal. These observations imply that classical tumors also have more clearly established cell polarities as compared to basal tumors.
- Proteomic analysis revealed that several cell polarity proteins, including CGN and SPTBN1, are much more phosphorylated in classical compared to basal tumors. Decreased cell polarity has been found to play a role in promoting cancer metastasis and tumor growth.³

Methods

Overall Project Overview

- Create overexpression constructs
- Transfect mutant construct
- CRISPR
- Validate knockouts
- Assess cell polarity
- Assess cell viability

Immunofluorescent staining pre-knockout

1. PDX Organoid establishment:
   Patient derived xenografts Human tumors were transplanted into immunocompromised mice to create patient derived xenograft (PDX) tumors. The PDX tumors were then harvested and cultured into organoids.

2. Immunofluorescent staining:
   Organoids were plated on Matrigel domes. The organoids were then fixed, permeabilized, blocked and incubated with primary and secondary antibodies and DAPI. Primary antibody only and secondary antibody only negative controls were used for each staining test, along with a positive cell line control.

3. Immunofluorescent imaging:
   Images were taken with Keyence microscope in brightfield, DAPI (blue fluorescence that stains for DNA) and either mCherry (red) or GFP (green). mCherry and GFP exposure was adjusted in test lines according to negative controls.

Effects of cingulin and SPTBN1 protein knockout on pancreatic ductal adenocarcinoma tumor cell polarity and subtype

CGN and SPTBN1 not visible in wild-type Classical organoids

- CGN and SPTBN1 proteins are involved in maintaining cell polarity and tumor growth.

Results

- SPTBN1 plasmid validated

Conclusion

- SPTBN1 and SCRIB had low signal in (+) HEK293T control, but none in organoid.
- CGN had strong 2d cell line (+) control signal but none in organoid.
- Possible solutions: Choose better +/- control lines for SPTBN1, and SCRIB. Use CGN controls in Matrigel. Do an siRNA knockdown as a negative control.
- Developed Bravo Robot protocol that can plate organoid domes for growth assays.
- We ordered and have received the 3-point mutated exogenous construct in lentiviral vector of cingulin and are waiting for the SPTBN1 construct.

Future Studies and Directions

- Roles of other cell polarity proteins in PDAC.
- Stain with better SPTBN1 positive and negative controls.
- Stain with different organoid lines and types and concentrations of primary antibody
- Perform growth assays on CGN and SPTBN1 wildtype and knockout organoids

References


Acknowledgements

Dr. Jen Jen Yeh, Sandy Zarmer, General Biosystems Inc. Lineberger Cancer Center, UNC-CH