Mitochondrial Phenotypes and β-II Spectrin Expression in Cancer Cells as a result of TGF-β Induction

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) accounts for about 3% of all cancers and is responsible for about 8% of all cancer deaths in the United States. PDAC is an aggressive malignancy characterized by extensive metabolic reprogramming, cytoskeletal remodeling, and dysregulated signaling pathways that together drive its rapid progression and poor clinical outcomes. Because of this, PDAC is rarely found in early stages when the chance of curing is at its greatest. Currently, there is one biomarker for PDAC detection, called CA19-9, a sugar molecule that is found on the surface of some cancers. CA19-9 is also elevated in non-cancerous conditions like liver disease, gallstones, or pancreatitis. Some people with pancreatic cancer may not make CA 19-9 making it even harder to use this as a proper biomarker. Spectrins, including βII-spectrin (SPTBN1), form a flexible lattice-like scaffolding network that helps maintain cellular architecture, anchor organelles, and coordinate signaling events.

Given βII-spectrin's role as both a structural support protein and a mediator of TGF-β/SMAD signaling, we aimed to determine how changes in its expression influence mitochondrial integrity in PDAC cells and whether βII-spectrin associated signaling (up or downstream) events may serve as potential biomarkers for early PDAC detection. Following one hour of TGF-β treatment, PDAC cells exhibit striking mitochondrial collapse, consistent with acute activation of stress pathways that disrupt mitochondrial dynamics and elevate reactive oxygen species. βII-spectrin staining appears diminished and less robust relative to untreated or normal PDAC cells, suggesting that TGF-β signaling triggers spectrin network destabilization or redistribution. This reduction in βII-spectrin may compromise the spectrin lattice's ability to support mitochondrial structure, thereby sensitizing organelles to deformation under stress. Together, these findings indicate that βII-spectrin is a key regulator of mitochondrial stability in PDAC and highlight its upstream and downstream signaling effects as a promising avenue for identifying possible new PDAC biomarkers.

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Table of Contents

Abstract **2**
Acknowledgements 3
Table of Contents **4**
Introduction **5**
Global Impact 6
Risk Analysis 6
Materials and Methods **7**
Materials 7
Instruments 7
Methods 8
Results **9**
Discussions **9**
Conclusions **10**
Literature Cited **11**

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and one of the deadliest. Most people diagnosed with PDAC end up passing away from this disease, with a mortality to incident rate of around 1 (Kleeff et al., 2016). There are two different types of pancreatic cancer: PDAC starts in the duct cells of the pancreas (which help carry digestive enzymes), and pancreatic neuroendocrine tumors (PanNETs), which come from the hormone producing cells within the pancreas. PanNETs are the less common form of pancreatic cancer, they normally grow slower, but they can still become aggressive and metastasize. Symptoms are noticeable in PDAC until it is in advanced stages. Symptoms like jaundice, stomach pain, weight loss, and fatigue will manifest. At that point, around 85-95% of cases cannot be fixed with surgery or chemo (Rawla et al., 2019). Research has found that PDAC is frequently associated with genetic mutations in SMAD4, a gene that is a key piece of the TGF-β signaling pathway (tumor suppressor). Proteins like β-II spectrin intrigue scientists because they play a huge role in the signaling pathway, and having low levels of this protein could potentially show up in the blood before the cancer reaches advanced stages. This makes TGF-β proteins a promising area to find new biomarkers to help detect pancreatic cancer earlier. A specific protein is β-II spectrin (SPTBN1). This protein is part of the cell's structure, keeps the cell stable, and helps support tumor-suppressing signals. β-II spectrin works hand in hand with the TGF-β/Smad pathway by helping Smad proteins get to the correct places so the TGF-β can help stop elevated cell growth (Zhang et al., 2017). When β-11 spectrin levels are reduced/low, the pathway breaks down, leading to uncontrolled cell growth, unstable DNA, and cancer progression. There have also been studies that show β-11 spectrin plays a role in the mitochondria's health. The mitochondria manages cell death and energy production. In many kinds of cancer, low levels of β-11 spectrin are connected with negative prognosis. This makes β-11 spectrin an important tumor suppressor and a regulator of cell health. Testing for B-11 in blood could be a new way of catching pancreatic cancer earlier and treat patients sooner.

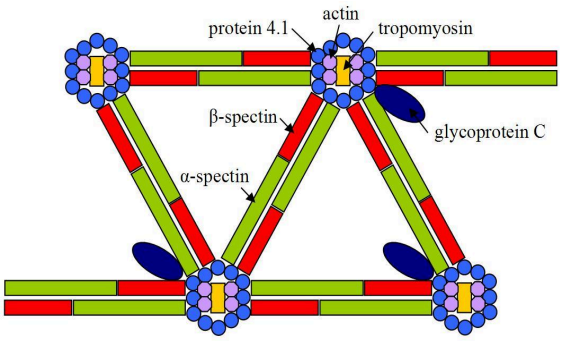

Figure 1
Diagram of cell membrane. Spectrin proteins connect at actin points to form a supportive structure. Proteins like 4.1 tropomyosin, and glycoprotein C help anchor the structure to membrane, keeping stability and shape in the cell

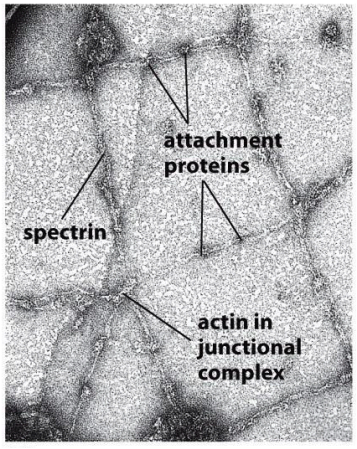

Figure 2
Microscope image showing cells structural network. Spectrin proteins create a lattice pattern and connect at actin junctions through attachment proteins.

Global Impact

Cancer is one of the main causes of death around the world, and pancreatic cancer can be even more deadly because it's usually caught in the later stages. There aren’t many clear symptoms until the cancer is in later stages, so there's not a lot of treatments available and survival chances are low (Siegel et al., 2024). Catching a pancreatic tumor before it turns cancerous can make a huge difference, but unfortunately there aren't a lot of tests/markers that can catch it this early. Finding a new blood-based biomarker is incredibly important, and could make early detection easier and less invasive.

Risk Analysis

This study utilized PANC-1 cancer cells (human cell line), obtained from the lab's personal liquid nitrogen tank. Cells were cultured in Dulbecco’s Modified Eagle Medium with 10% Fetal Bovine Serum. Immunofluorescence staining was performed to visualize mitochondrial structure and β-II spectrin levels, with imaging conducted under a UV light. All experimental work was performed in a biosafety level 2 (BSL-2) hood under the supervision of a mentor. Sterile technique and appropriate personal protective equipment (PPE) were required to prevent contamination of samples. OSHA certification and University of Arizona laboratory safety training were completed prior to participation.

Materials and Methods

Materials

This study used the human pancreatic cell line Panc1 (wildtype). Cancer cells were cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Transforming Growth Factor Beta (TGF-β) was used as a treatment and a pathway activation in the cells. Phosphate-buffered saline (PBS) and trypsin-EDTA were used for washing and transferring cells. Cells were fixed using 4% paraformaldehyde (PFA) prior to staining. To visualize cellular structures, a mitochondrial stain and antibody targeting βII-spectrin were used, followed by a fluorescent antibody. Personal protective equipment (PPE) included lab coats, gloves, and goggles which were worn during all procedures.

Instruments

Standard cell culture supplies included culture flasks, plates, micropipettes, micropipette tips, glass slides, and coverslips. All cell culture procedures were performed in a biosafety cabinet (level 2) to maintain sterile conditions. Cells were grown in a CO_2 incubator at 37°C. A centrifuge was used during cell processing steps. For imaging and analysis, a fluorescence microscope was used for imaging of mitochondrial morphology and β-II spectrin localization. Image analysis and fluorescence quantification were performed using ImageJ. Cancer cells were stored in labs personal liquid nitrogen tanks for preservation. Additional lab equipment includes a refrigerator, freezer, and a water bath for reagent preparation.

Methods

This study will investigate the role of βII-spectrin in pancreatic cancer cell lines using live-cell imaging, cell culture, and immunofluorescence staining. PANC-1 cells will be maintained under standard culture conditions and routinely monitored for viability, with adherent cells passaged at 80–90% confluency. Cell numbers for seeding will be calculated based on viability counts using Trypan Blue and an automated cell counter, and media master mixes will be prepared for consistent plating across experimental wells. To examine mitochondrial function and βII-spectrin localization, cells will be stained with MitoTracker Red and immunolabeled, “co-staining”, for βII-spectrin following fixation, permeabilization, and blocking. Stained cells will be counterstained with DAPI and mounted on glass slides using antifade media for imaging. Fluorescent signals will be captured using appropriate microscope channels (DAPI = blue, AF488 = green, AF594 = red).

To assess the effects of TGF-β signaling, cells will be treated with either no TGF-β (control), low TGF-β, or high TGF-β for 16–18 hours before fixation and staining. Low and high treatment doses are designed to determine the minimal effective concentration that modulates mitochondrial and βII-spectrin responses while avoiding cellular toxicity. Following imaging, corrected total cell fluorescence (CTCF) will be quantified for both mitochondrial and βII-spectrin signals using ImageJ, with background fluorescence subtracted for each image. These measurements will then be used to generate comparative graphs in GraphPad Prism, allowing visualization and statistical comparison across treatment conditions.

Data analysis will focus on fluorescence intensity and mitochondrial morphology across experimental groups. Statistical tests, including ANOVA or t-tests, will evaluate differences between treatment groups, with significance set at $p < 0.05$. All experiments will be conducted under standard biosafety conditions, including ethanol disinfection of hoods and surfaces, proper handling of trypsin and paraformaldehyde, and appropriate storage of reagents. Detailed step-by-step protocols will be included for reproducibility. All experiments performed under standard biosafety conditions: Detailed step-by-step protocols – under supervision always.

Results

Immunofluorescence imaging showed notable changes in the mitochondrial structure, and β-II spectrin localization in Panc1 WT cells. The mitochondria's shape and organization was altered, indicating cellular stress. There was reduced expression in β-II spectrin. These results suggest cytoskeletal instability and collapsed mitochondria. Both of these processes are associated with TGF-β pathways in pancreatic cancer cells. This data was collected over a 2 week period.

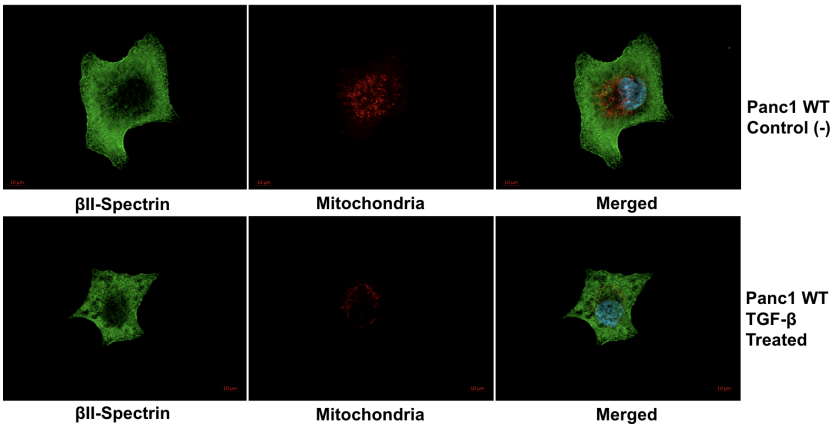

Figure 3

Fluorescent images of Panc1 WT cells show β-II spectrin (green), mitochondria (red) and the merged images. Control cells (top) have a more organized structure, and a visible mitochondria. Treated cells (bottom) show changes in mitochondrial structure and β-II spectrin.

Discussions

βII-spectrin acts as an anchor for SMAD3 in the cytoplasm. Its key roles include:
maintaining plasma membrane and organelle integrity, as well as regulating TGF-β/SMAD trafficking and transcriptional responses. When βII-spectrin is reduced, the cytoskeleton weakens, which can disrupt mitochondrial positioning and stability. This explains why altered mitochondrial morphology appears. The mitochondrial collapse is likely an after effect of cytoskeletal destabilization.

TGF-β signaling can decrease βII-spectrin stability possibly by proteasomal degradation triggered by SMAD-dependent feedback loops or through phosphorylation and cytoskeletal remodeling, leading to

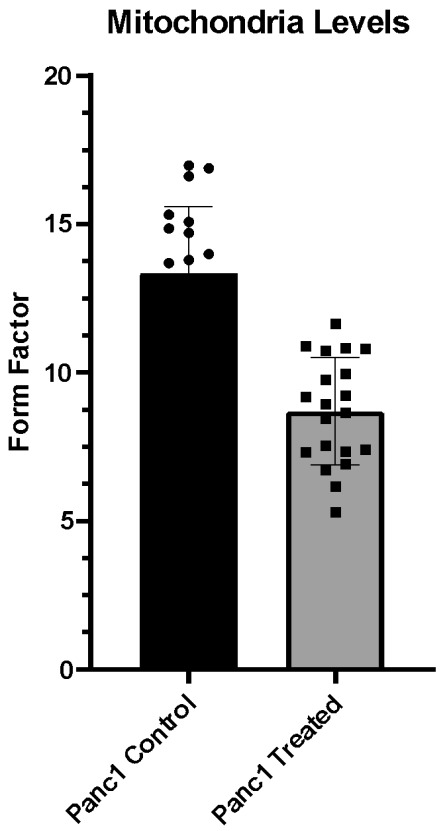

Figure 4

Graph from ImageJ showing fragmented or condensed morphology of mitochondria. The form factor is a quantitative morphological measure used to assess mitochondrial shape.

redistribution or weakening of the networks. TGF-β signaling plays a key role in pancreatic cancer, it serves as a tumor suppressor, but later on serves as a tumor promoter. If βII-spectrin levels decrease, it's likely it will enhance abnormal TGF- β signaling. Cytoskeletal instability may contribute to invasiveness, metastatic potential, and increased cell migration.

Limitations of this study include small sample sizes, analysis limited to Panc1 WT cells, and primarily image based observations.

Conclusions

TGF-β rapidly induces mitochondrial and cytoskeletal stress in pancreatic cancer cells through βII-spectrin destabilization. Disruption of βII-spectrin weakens cytoskeletal integrity and is associated with altered mitochondrial morphology, which suggests a functional connection between mitochondrial stability and mitochondrial health. βII-spectrin has a dual as a structural scaffolding as well as a mediator of TGF-β signaling. This project investigated how changes in βII-spectrin affect mitochondrial integrity and if it could serve as an early PDAC biomarker. One hour of TGF-β treatment, the Panc1 cells displayed mitochondrial collapse. Severe mitochondrial collapse can impair ATP production, and signal apoptotic signaling pathways, ultimately reducing cancer cell viability. βII-spectrin is reduced and less organized when compared to untreated cells, suggesting that TGF-β signaling triggers spectrin destabilization. Loss of βII-spectrin weakening cytoskeletal support and sensitizing mitochondria to deformation under stress, highlights its role as a regulator of mitochondrial stability in PDAC.

Early biomarker potential:

Reduced βII-spectrin expression or redistribution could serve as an early marker of TGF-β pathway activation in pancreatic tumors. Combined imaging of mitochondrial morphology and βII-spectrin integrity could provide a functional readout of TGF-β sensitivity

Therapeutic insight:

In advanced disease (where TGF-β promotes invasion), stabilizing βII-spectrin or blocking TGF-β signaling might suppress metastasis.

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