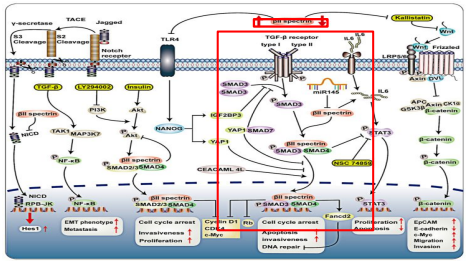
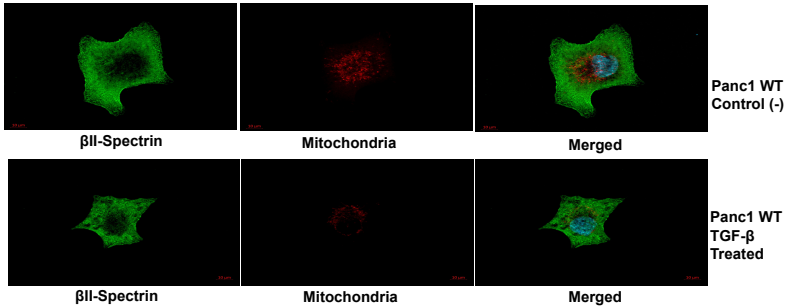
Research Question

How does TGF-β treatment affect βII-Spectrin expression and mitochondrial integrity in pancreatic cancer cells?



Data Analysis & Results

TGF-β and βII-Spectrin needed for Mitochondria Integrity



Methods

Cell Culture:
- Panc1 Cancer cell line seeding for 70-80% confluency on glass immunofluorescence slides
- MitoTracker Staining 100nM

Treatment 1 hour:
- No TGF-β (Control)
- TGF-β (1µl/ml)

Assess interaction between mitochondrial and βII-Spectrin avoiding cell toxicity

Immunofluorescences:
- MitoTracker Red labeling the mitochondria
- βII-Spectrin antibody (1:250) Green Mouse immunolabeling location on cell

Quantification:
- ImageJ analysis
- Corrected Total Cell Fluorescence (CTCF)
- GraphPad Prism data visualized
- Fluorescence intensity + Mitochondrial morphology

Microscope Channels:
DAPI = blue
AF488 = green
AF594 = red

All experiments performed under standard biosafety conditions: Detailed step-by-step protocols – under supervision always

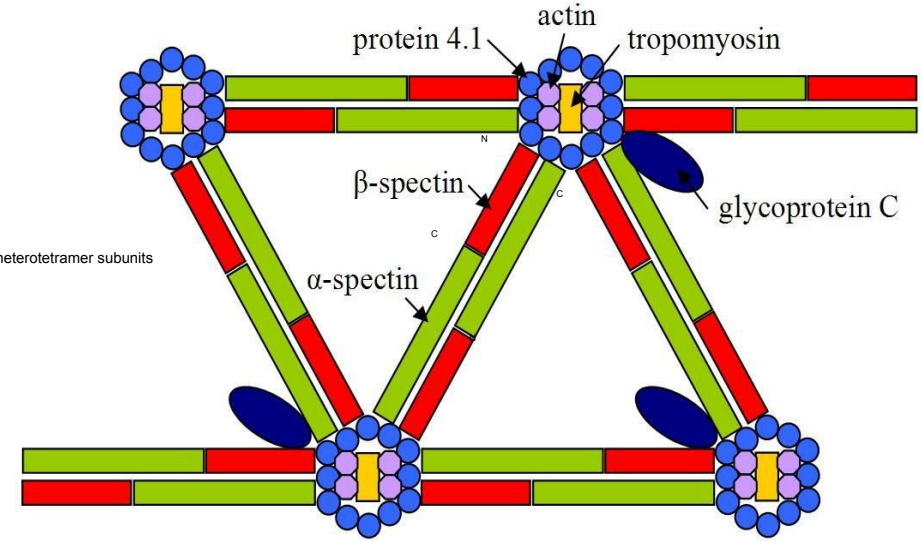
Interpretation & Conclusions

TGF-β rapidly induces mitochondrial and cytoskeletal stress in pancreatic cancer cells through βII-spectrin destabilization.

➔ Early biomarker potential

➔ Therapeutic Insight: In advanced disease (where TGF-β promotes invasion), stabilizing βII-spectrin might suppress metastasis

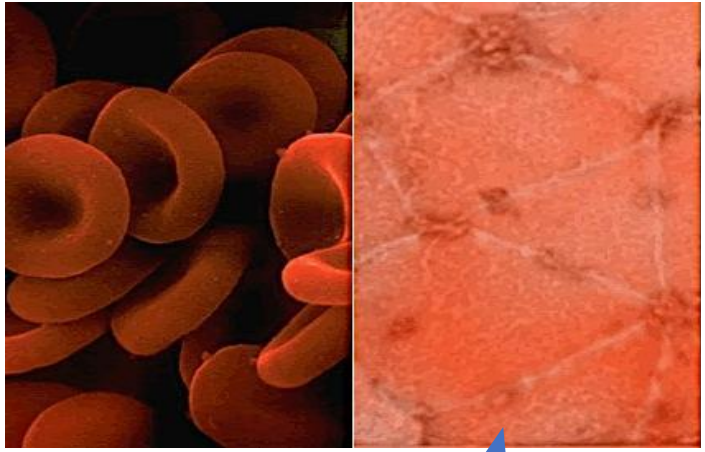
Introduction



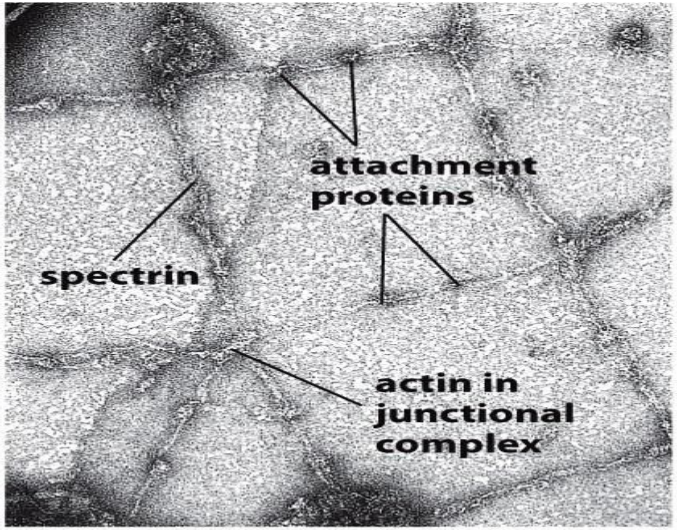
α, β- spectrin heterotetramer subunits

- Spectrin cytoskeletal network (lines inner surface of cell membrane)
- α-spectrin and β-spectrin link together to form flexible support scaffolding
- spectrin strands connect at actin junctions
- Helps maintain cell shape and stability

Introduction



- First discovered in red blood cells
- Lattice shape



- Spectrin cytoskeleton beneath cell membrane
- Attachment proteins anchor network to membrane
- Actin junction connect spectrin filaments

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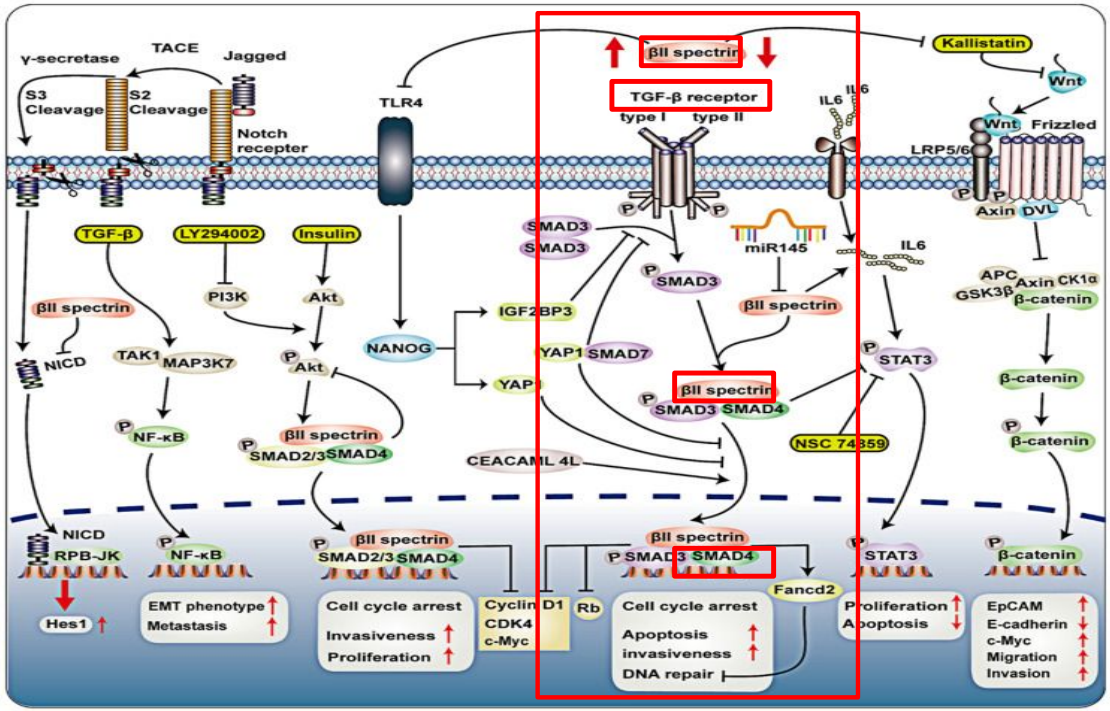

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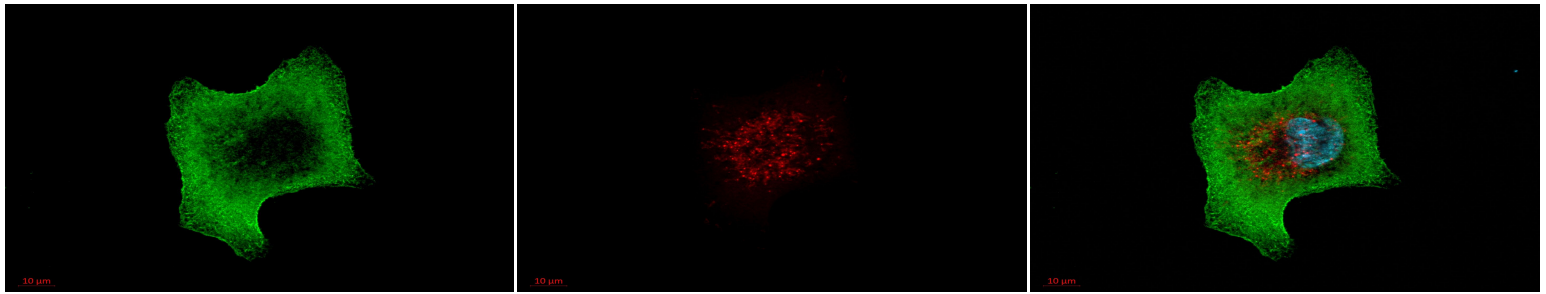
Results

TGF-β and βII-Spectrin needed for Mitochondria Integrity



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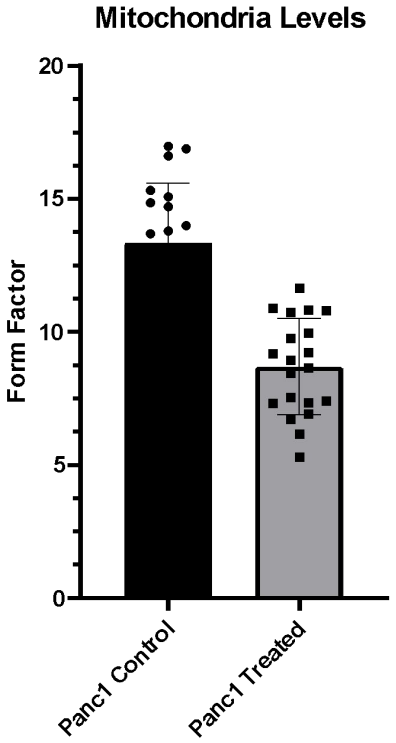
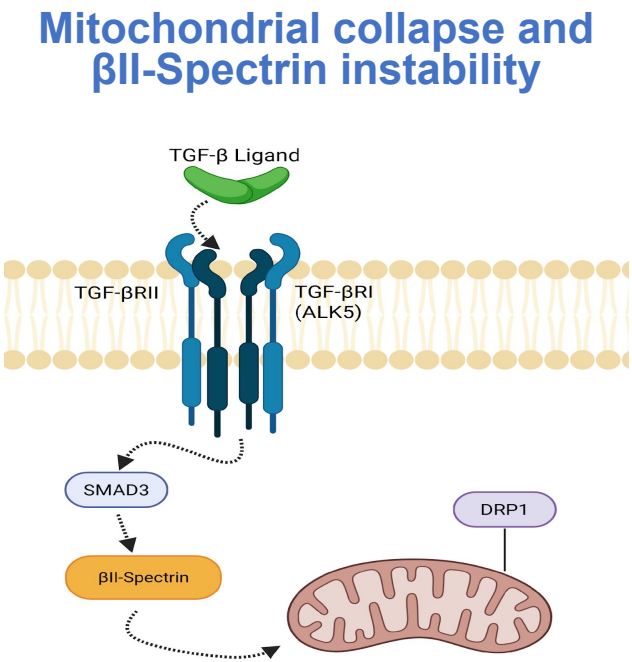


- Using ImageJ, the form factor is a quantitative morphological measure used to assess mitochondrial shape and is defined by the formula:

Form Factor = $\frac{p^2}{4 \times \pi \times a}$

- Fragmented or condensed morphology, often seen under stress or depolarization
- A sign of mitochondrial fission or loss of membrane potential
- Within 1 hour, TGF-β can initiate a stress response that alters mitochondrial dynamics and causes them to appear “collapsed” or fragmented

Discussion



- βII-spectrin acts as an anchor for SMAD3 in the cytoplasm.

Key roles:
-Structural: Maintains plasma membrane and organelle integrity.
-Signaling: Regulates TGF-β/SMAD trafficking and transcriptional responses.

- TGF-β signaling can decrease βII-spectrin stability possibly by:

-Proteasomal degradation triggered by SMAD-dependent feedback loops.
-Phosphorylation and cytoskeletal remodeling, leading to redistribution or weakening of the networks

Ideas for future research

TGF-β rapidly induces mitochondrial and cytoskeletal stress in pancreatic cancer cells through β2-spectrin destabilization.

✔ 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 β2-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.

References

- Yang P, Yang Y, *et al.* βII spectrin (SPTBN1): biological function and clinical potential in cancer and other diseases. ***Int J Biol Sci.*** (2021)
- Flores PC, Ahmed T, **Podgorski J**, *et al.* Phosphoproteomic profiling identifies DNMT1 as a key substrate of beta IV spectrin-dependent ERK/MAPK signaling in suppressing angiogenesis. ***Biochem Biophys Res Commun.*** (2024)
- Kwak EA, Pan CC, *et al.* βIV-spectrin as a stalk cell-intrinsic regulator of VEGF
- signaling. ***Nat Commun.*** (2022)