Confirming The Effects of FOXN2 mRNA Expression On LMLN, C9orf116, and LRRC6

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Introduction

Pancreatic cancer is the 3rd deadliest type of cancer in the United States (Yi-Jin, 2017). Pancreatic cancer is often diagnosed late stage due to its late onset of symptoms and rapid growth rates. Minimal research has been conducted about the genes involved in pancreatic cancer, however, mutations in certain genes are known to play a role in some cancers by causing cells to fail cell cycle checkpoints. Research done by the Johnson Lab has targeted FOXN2 as having a role in pancreatic cancer. During experimentation it was shown that FOXN2 regulates the expression of LMLN, C9orF116, and LRRC6 (Johnson, year). Genetically modifying FOXN2 allows for analyzing protein levels via a western blot between control PANC-SCR cells and modified PANC-FOXN2 cell lines. Its thought that FOXN2 plays a role in pancreatic cancer by lowering the expression of LMLN, LRRC6, and C9orf116 (Santibanez, 2019).

Methodology

A Western Blot was performed using 6 lysate samples; PANC-SCR 1, PANC-SCR 2, PANC-SCR 3, PANC-FOXN2 1, PANC-FOXN2 2, and PANC-FOXN2 3.

- A BCA assay was performed to obtain sample concentration (Fig. 1).
- The gel was run and transferred to a sensitive membrane where it soaked in a primary antibody bath (Fig. 2)
- The membranes were washed in a 10x TBST solution
- The membrane was then coated in a secondary antibody that attaches to the primary
- Chemiluminescent dye was used so that LMLN and C9orf116 levels can be visualized

Western Blot

Figure 1.

Performing the BCA assay to determine the amount of sample needed.

Figure 2.

Gel running in preparation for sensitive membrane transfer.

Protein Scanner

Figure 3.
LRRC6 showing small faint bands under the protein scanner.

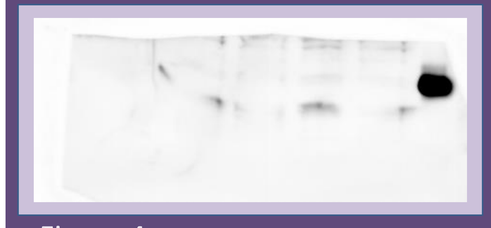

Figure 4.
C9orf116 showing small faint bands under the protein scanner.

Results

The images from the protein scanner revealed that 2 of the 3 proteins were able to be visualized. LRRC6 (fig 3.) and C9orf116 (fig. 4) were seen with faint small bands, indicating that expression could be low in the sample. LMLN was not able to be observed through the scan.

Conclusion

After visualizing the protein levels of LRRC6 and C9orf116, results showed that the genes are not differentially expressed between control and modified cell lines. This doesn't support the original hypothesis as it was expected that the modified cells would have higher expressivity.

LMLN may need to be tested further with new antibodies that may bind more effectively to the protein of interest. Another potential source of error could be that the amount of protein may be too small, making it difficult for the protein scanner to pick up on the protein levels.

Source of errors should be addressed, and further research should be conducted before concluding weather or not FOXN2 influences the expression of LMLN, LRRC6, and C9orf116.

References

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