VERIFYING ALTERED GENE EXPRESSION IN PANCREATIC CANCER USING BIOTECHNOLOGY

LAURYN DAVIS, KRISTEN C. JOHNSON, PHD, BETH WILKES
DEPARTMENT OF NATURAL SCIENCES

INTRODUCTION

Pancreatic cancer arises from uncontrolled cell growth and division when tumor suppressor genes and oncogenes are mutated [1]. In pancreatic cancer, FOXN2 is upregulated in the late stages and appears to act as an oncogene, thus promoting cancer progression and malignancy [2]. When protein levels were quantified with RNAseq and RT-qPCR data, it was found that the expression of FOXN2 when downregulated caused higher mRNA levels of the proteins LMLN, C9orf116, and LRRC6. Using a laboratory technique called western blot, protein levels of the three proteins can be confirmed.

METHODS

The protein lysate samples used in this experiment were extracted from the cell lines Panc-scr, the control cells, and Panc-FOXN2, the knockdown FOXN2 cells.

BCA PROTEIN ASSAY

Samples were placed in a spectrophotometer to obtain absorbance levels. Using an excel file and standard curve (Figure 1) the concentrations of each sample were determined.

WESTERN BLOT

Using the data from the BCA Assay, a western blot was performed (Figures 2,3,4). The three genes LMLN, C9orf116, and LRRC6, were observed under a chemiluminescence imaging system (figure 5,6).

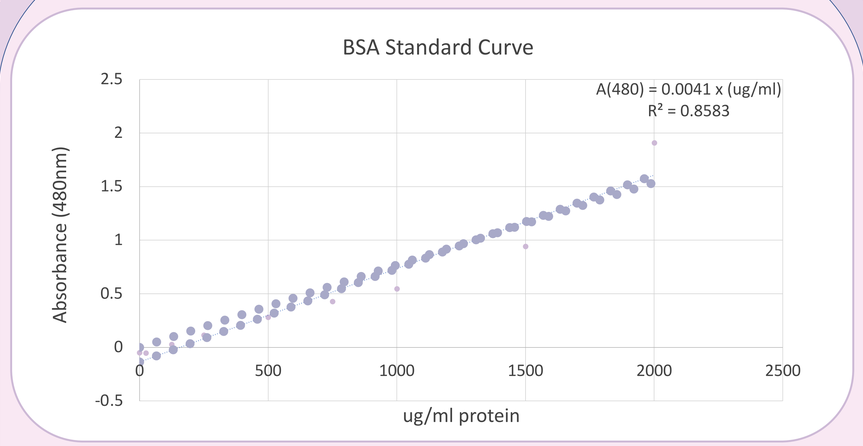

Figure 1. Standard curve of expected concentrations and equation that was used to determine the concentrations of each sample.

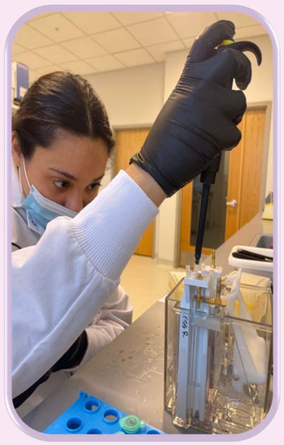

Figure 2. 50µL of the samples Panc-scr4, Panc-scr5, Panc-FOXN2-4, Panc-FOXN2-5 were pipetted into a 12% gel.

Figure 3. A 12% gel with the samples Panc-scr4, Panc-scr5, Panc-FOXN2-4, Panc-FOXN2-5.

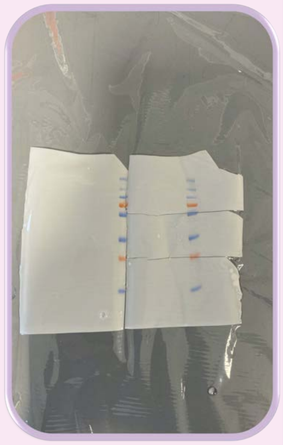

Figure 4. Using known weights of the proteins in kDa's and referencing the ladder, the sensitive membrane was cut based on the size of each protein.

RESULTS

Figure 5. A chemiluminescence image of the samples Panc-scr4, Panc-scr5, Panc-FOXN2-4, Panc-FOXN2-5 using the antibodies LRRC6 and Actin.

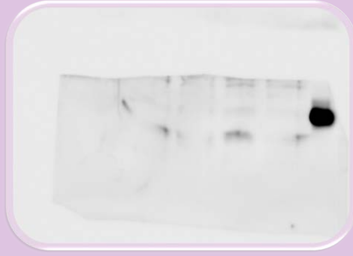

Figure 6. A chemiluminescence image of the samples Panc-scr4, Panc-scr5, Panc-FOXN2-4, Panc-FOXN2-5 using the antibody C9orf116.

CONCLUSION

The western blot results (Figures 5, 6) show there is no difference in expression between the Panc-scr cells and the Panc-FOXN2 cells of the proteins LRRC6 and C9orf116. It was expected to see an increase in the expression of the proteins LMLN, LRRC6, and C9orf116 [2]. The results for the protein LMLN were unobtainable, and the reason for this is not certain. Possible explanations for this could be the antibody was ineffective, or low protein levels in the samples made LMLN not visible in the western blot. The results did not support the initial data collected from an ASPC1 RNASeq and a RT-qPCR.

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[1] American Cancer Society. (n.d.). *Pancreatic cancer*. American Cancer Society. Retrieved March 29, 2022, from https://www.cancer.org/cancer/pancreatic-cancer.html
[2] Santibanez B, Oliveira S, Duggan K, Lu K, Johnson K. Abstract C49: Knockdown of FOXN2 enhances adhesion and reduces migration in pancreatic cancer cells. Cancer Res. 2019;79(24 Supplement):C049-C049. doi:10.1158/1538-7445.PANCA19-C049