Pseudouridine Synthase mRNA and Protein Expression Levels in Gastric Cancer

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Introduction

- GC is the most common cause of death related to malignancies worldwide after lung cancer.[1]
- General decline in GC rates due to improvement in the refrigeration of foods and a decrease in *H. pylori* infections, but it remains an imperative and pressing issue within current research.[2]
- One type of post-translational RNA modification, where uridine is converted into pseudouridine (ψ) via an enzyme called pseudouridine synthase (ψ-synthase), may be connected to cancer.[3]
- By collecting tissue samples of over 100 gastric cancer patients and measuring the protein and mRNA expression levels of the gastric cancer cells, we hope to narrow down and provide direction to the most relevant ψ-synthase of GC to further investigate.

Objective

Our goal in this investigation is to select candidates for further investigation from a pool of genes that code for ψ-synthase (*PUS7, PUSL1, PUS3, DKC1, PUS1, TRUB1, RPUSD1, PUS7L, RPUSD3, TRUB2, RPUSD4, RPUSD2,* and *PUS10*). The main criterion for selection is both abnormal mRNA and protein expression levels.

Methods

- Over 100 gastric tissue samples along with corresponding normal tissue samples were collected from patients in different stages of gastric cancer at nearby hospitals. Samples were then stored in a N_2 container until needed for RNA recovery.
- Within approximately two months, the RNA from these tissue samples were collected at a rate of about 20 samples per extraction, where each frozen tissue fragment was ground to fine powder on a mortar pre-chilled with N_2, then added to TRIzol reagent.
- The mRNA expression levels of the genes were then measured using quantitative reverse transcription PCR (RT-qPCR).
- Protein expression levels measured through a Western Blot.

Results

- Only *PUS7*, displays a significant difference between its normal and cancerous tissue, as a paired t-test on cDNA concentration results in a low p-value of 0.0004.
- However, Western Blot results show not a trend of protein expression, rather a variety of abnormalities for *PUS7* expression in cancerous gastric tissue (T) when compared to the stable gene, *ACTB* as well as its normal tissue counterpart (N) (*Figure 1*).
- In the leftmost protein column, *PUS7* T displays a higher concentration; in the middle column, the concentration of PUS7 T is significantly higher than its normal counterpart, while PUS7 T in the rightmost column shows a lower, almost nonexistent concentration than *PUS7* N.

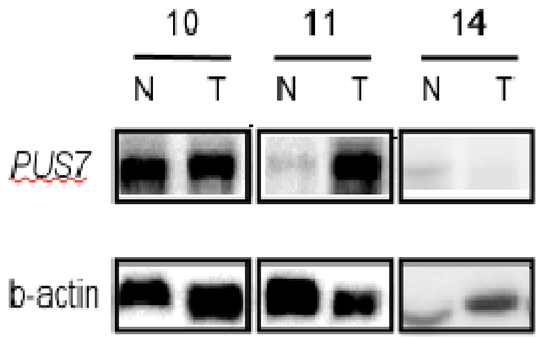

Figure 1. Protein expression levels of PUS7 *compared to stable gene* ACTB. *The top numbers are the amount of each sample loaded In microliters.*

Discussion and Conclusion

- Based upon our results of both qPCR and Western Blot, we observe abnormal mRNA expression levels and fluctuating protein expression levels in *PUS7* from gastric tissue, qualifying *PUS7* as a candidate for subsequent investigation.
- For future investigations into *PUS7,* we suggest measuring protein levels and mRNA levels of PUS7 in various cancer cell lines (MKN45, AGS, GES-1, BGC, HGC, MGC, SGC, WP6, N87) as well as gastric cancer tissue from patients.
- Additionally, knockdown and knockout investigations of *PUS7* in gastric cancer cell lines may be necessary to elucidate the functional effects of *PUS7*.

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[1] Sitarz, R., Skierucha, M., Mielko, J., Offerhaus, G., Maciejewski, R., & Polkowski, W. P. (2018). Gastric cancer: epidemiology, prevention, classification, and treatment. *Cancer management and research, 10*, 239–248. doi:10.2147/CMAR.S149619
[2] Ibid.
[3] Penzo, M., Guerrieri, A. N., Zacchini, F., Treré, D., & Montanaro, L. (2017). RNA Pseudouridylation in Physiology and Medicine: For Better and for Worse. *Genes, 8*(11), 301. doi:10.3390/genes8110301