Selective Synthetic Lethality of Colon Cancer Cells via CRISPR-Cas9 Mediated Knockout

Ibnat Meah, College of Arts & Sciences 2025
Eric Brown, Department of Cancer Biology, Perelman School of Medicine

Abstract

WRN is a RecQ DNA helicase that is a lethal target for cancer cells with microsatellite instability (MSI), a mutation that results in impaired mismatch repair. MSI cells that are depleted of WRN undergo cell cycle arrest and/or apoptosis due to DNA double-strand breaks. Previous iPOND experiments conducted by the Brown Lab found multiple proteins were recruited to the replication fork following WRN depletion in cells. This project chose to knockout one of the recruited proteins, referred to as geneX, in the human colon cancer cell line RKO. This was done via the CRISPR-Cas9 mechanism. We hoped to observe a synergistic effect between WRN and GeneX in cancer cell death as measured by flow cytometry. The results found that the knockout of solely GeneX in RKO cells results in cell death. We also did not observe a synergistic effect between WRN and geneX suggesting that geneX itself is a lethal target for RKO cells. These findings support the development of therapeutic agents that target specific genes in MSI cells.

Introduction

Colorectal is the third most common cancer diagnosed in the United States. It is also the third leading cause of cancer-related deaths in men and in women. Colorectal cancer has a 5-year survival rate of 65.1% and 151,030 adults in the United States are estimated to be diagnosed with colorectal cancer this year [1]. Current treatments for colorectal cancer includes surgery, chemotherapy, and radiation. However, these therapeutics are limited in preventing the progression of the disease and attacks both cancerous and healthy cells. Thus, more targeted therapeutics are needed. Advances in human genome studies have found that gene alterations are key in the treatment of cancer.

One such gene alteration that has proved to be lethal for cancer cells is the depletion of WRN. WRN depletion creates double stranded breaks in MSI cells as TA-dinucleotide repeats undergo large-scale expansions as they are highly unstable in MSI cells. These expanded TA repeats create non-B DNA secondary structures such as G quadraplexes, cruciform, triplexes, and tetraplexes. Formation of these structures stall the replication forks which in turn activates the ATR checkpoint kinase. These non-B DNA secondary structures require unwinding by the WRN helicase. When MSI cells are depleted of WRN, the TA repeats are vulnerable to cleavage by the MUS81 nuclease which leads to massive chromosome shattering [2].

Here, we used CRISPR-Cas9 to knockout geneX in RKO cells with the objective of observing a synergistic effect between WRN and geneX in increased cancer cell death. In the CRISPR-Cas9 mechanism a user defined guide RNA (sgRNA) targeting the gene of interest and the Cas9 protein combine to form a Cas9:sgRNA complex. This complex then recognizes and binds to the PAM sequence adjacent to the original target DNA and results in the unwinding of the DNA helix which then permits the guide RNA to pair with the target sequence. The Cas9 protein then cuts the DNA resulting in a double stranded break. Cells then employ various DNA repair mechanisms. However, these are highly error-prone and eventually results in the knockout of the target gene [3].

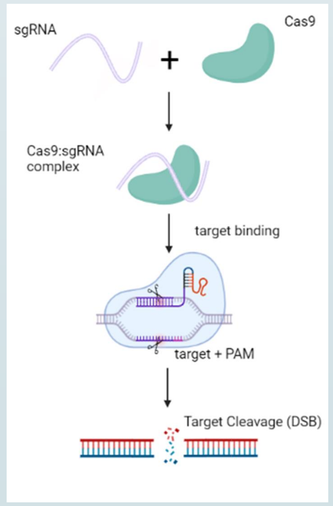


Figure 1: overview of CRISPR

Methodology

Initial Steps:

- sgRNA were designed using benchling
- the sense and antisense oligo of the sgRNA were annealed in a PCR tube
- LRG 2.1T plasmid was digested using BsmBI and the digested plasmid was run on a gel.
- the sgRNA oligo was ligated to the digested LRG 2.1T vector.

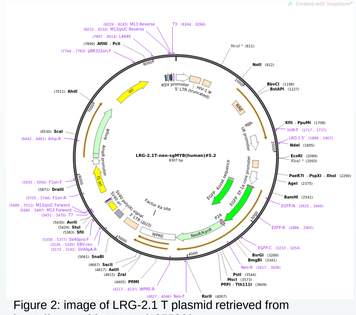


Figure 2: image of LRG-2.1 T plasmid retrieved from https://www.addgene.org/105592/

Transformation and Plating

Figure 4: overview of the transformation and plating process

- colonies were picked from the plates and grown overnight
- minipreps were then conducted on the colonies in order to isolate the plasmid DNA
- isolated plasmids were sent for Sanger Sequence verification of the correct sgRNA insert.

Lentiviral Transduction

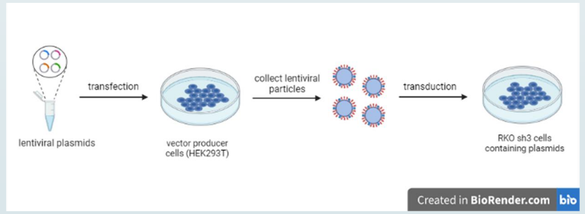


Figure 5: overview of the lentiviral transduction process

Flow Cytometry

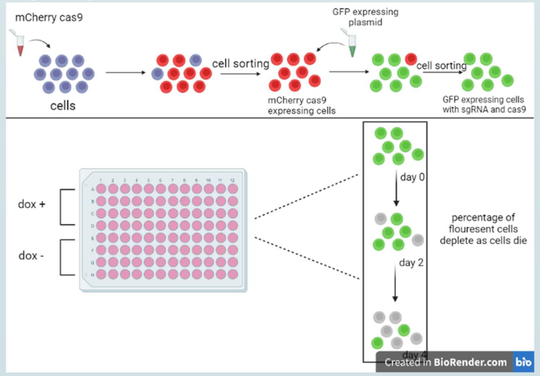
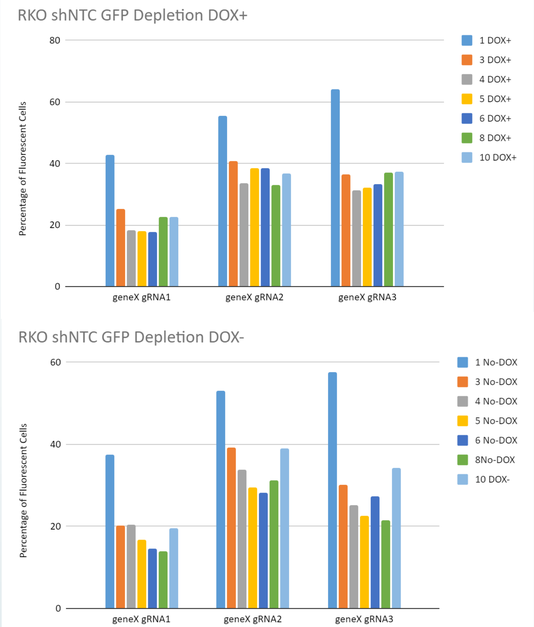


Figure 6: overview of flow cytometry

Results



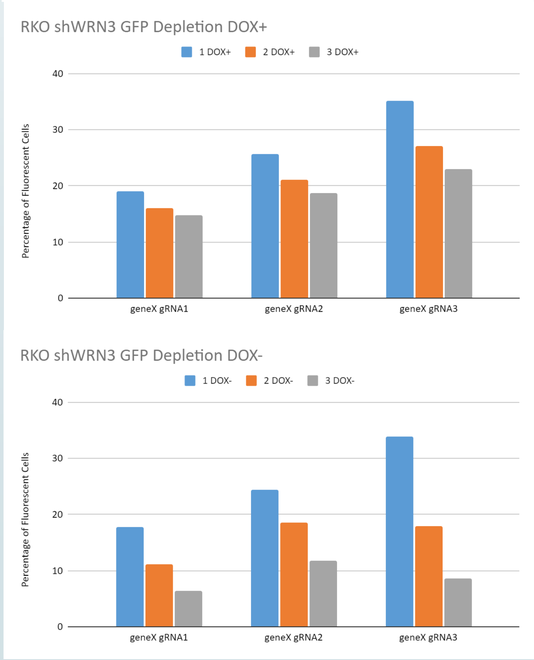


Figure 7: bar graphs depicting the results from the flow cytometry experiment

Conclusions

- the percentage of GFP fluorescent cells decreased in both the shNTC samples (where WRN was expressed) and the shWRN3 samples (where WRN was silenced).
- the percentage of GFP fluorescent cells also decreased in both the DOX+ (WRN is silenced) and DOX- (WRN is expressed) samples.
- the data does not support a synergistic relationship between WRN and geneX in RKO cell death as cell death still occurred when WRN was expressed
- the data indicates that geneX is a lethal target in RKO cells itself.

Future Directions

- Determine the mechanisms by which geneX knockout leads to death in RKO cells via immunofluorescence analysis
- Preform geneX knockout on microsatellite stable cells
- Apply results to a clinical setting via geneX inhibitors in mice models

References

[1] *Colorectal cancer statistics: How common is colorectal cancer?* American Cancer Society. (n.d.). Retrieved August 16, 2022, from https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html#:~:text=Deaths%20from%20colorectal%20cancer,about%2052%2C580%20deaths%20during%202022.

[2] van Wietmarschen, N., Sridharan, S., Nathan, W. J., Tubbs, A., Chan, E. M., Callen, E., Wu, W., Belinky, F., Tripathi, V., Wong, N., Foster, K., Noorbakhsh, J., Garimella, K., Cruz-Migoni, A., Sommers, J. A., Huang, Y., Borah, A. A., Smith, J. T., Kalfon, J., … Nussenzweig, A. (2020). Repeat expansions confer WRN dependence in microsatellite-unstable cancers. *Nature*, *586*(7828), 292–298. https://doi.org/10.1038/s41586-020-2769-8

[3] Feng Zhang, Yan Wen, Xiong Guo, CRISPR/Cas9 for genome editing: progress, implications and challenges, *Human Molecular Genetics*, Volume 23, Issue R1, 15 September 2014, Pages R40–R46, https://doi.org/10.1093/hmg/ddu125

Acknowledgements

I would like to thank Dr. Eric Brown for allowing to me work in his Cancer Biology Lab and for his support this summer. I would especially like to thank my mentor Dr. Muzaffer Kassab for all the the guidance he has provided me. I would also like to thank the other members of the Brown Lab for their continued support. Lastly, I would like to thank the University of Pennsylvania and the Penn Undergraduate Mentoring Program (PURM) for funding and supporting the opportunity to participate in undergraduate research.