Bioinformatic Analysis of Factors Recruited to the Replication Fork Following ATRi/WEE1i Treatment

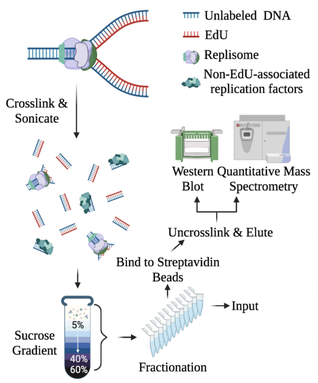
[1]**Alexandra Cordoba**, [1]Jasmine Peake, [1]Sydney Chanen, [2]Kasia Kulej, [1]Eric J. Brown

[1]University of Pennsylvania, Perelman School of Medicine, Department of Cancer Biology (Philadelphia, PA), [2]Children's Hospital of Philadelphia, Center for Childhood Cancer Research (Philadelphia, PA)

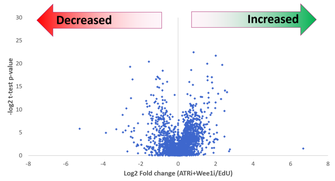
College of Arts and Sciences, Class of 2025

1. INTRODUCTION

A. iPOND: Isolation of Proteins On Nascent DNA

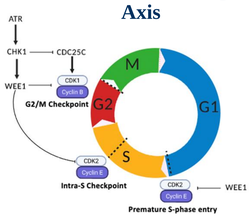


B. iPOND Data Output

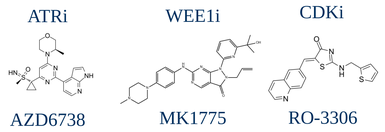


- iPOND is a technique that allows for the detection and quantification of proteins recruited to nascent DNA. We utilized a sucrose gradient to further increase resolution (1A).
- Following iPOND sample collection, data are analyzed via quantitative mass spectrometry and the data are presented in a volcano plot (1B).
- The ATR-CHK1-WEE1 Signaling Axis is essential in regulating the cell cycle especially during the premature S-phase entry, Intra-S checkpoint, and G2/M checkpoint (1C).
- The inhibitors applied are known to be involved in cell cycle regulation. This is essential considering that cell cycle inhibitors and regulators have high importance in cancer growth and development (1D).

C. ATR-CHK1-WEE1 Signaling Axis

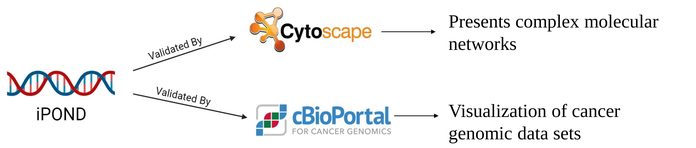


D. Inhibitors Known to be Involved in Cell Cycle Regulation

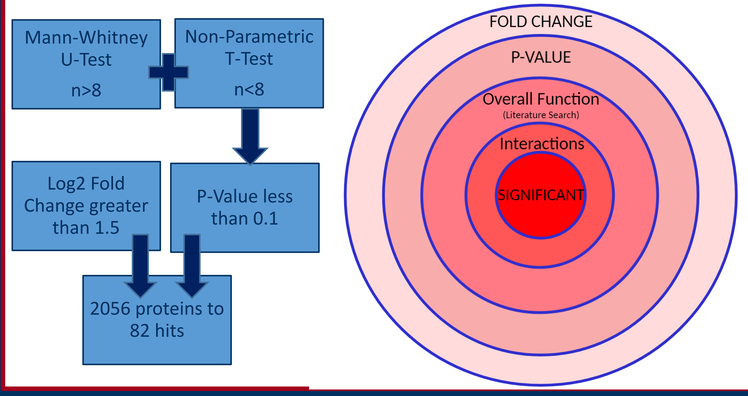


2. METHODS

A. Bioinformatic Analysis Overview



B. Prioritization Process in Identifying Hits



ABSTRACT

The ATR-CHK1-WEE1signaling axis is essential in cell cycle regulation and in maintaining cell cycle fidelity. ATR is a kinase that phosphorylates repair proteins and activates cell cycle arrest. Wee1 is a cell cycle inhibitor that activates CDK1 and CDK2 to initiate cell cycle arrest. Via low-dose combination treatment of ATRi and WEE1i we hope to develop a treatment for high grade serous ovarian cancers (HGSOCs). The factors that are recruited to the replication fork following drug treatment may be recruited to mitigate the effects caused by the drug. We are interested in these factors, as the treatment may be more effective if the patient has a deletion or truncation mutation in them. This furthers the efforts of personalized medicine where the treatments specifically match the cancer type to limit toxic effects. We employed iPOND to identify the factors that are recruited to the replication fork in response to the ATRi-WEE1i treatment. The iPOND-QMS process stands for the isolation of proteins on nascent DNA with quantitative mass spectrometry. This process is our strategy to determine which factors are present at the replication fork. The initial iPOND dataset made use of four different inhibitors ATRi, WEE1i, CDKi, and Aphidicolin. This project will focus specifically upon the proteins recruited under combination drug treatments including ATRi and WEE1i. **The bioinformatic analysis of such factors, provided by iPOND-QMS experimentation, under specific drug conditions to serve as a prioritization pathway for identifying hits is unresolved.** Seeking to validate the results of the iPOND-QMS experimentation and narrow down the list of 2056 proteins recruited to the replication fork, we hope to identify which proteins, if knocked down, would enhance the effects of the drug treatment. Specifically, Cytoscape, the STRING Database, and Cancer BioPortal are the main bioinformatic strategies employed throughout this project. Thus, through the validation of such data we hope to identify biomarkers for efficacious drug treatment. Additionally, we hope to gain a deeper understanding of the applications of bioinformatic analytical pathways, as well as developing a prioritizing process to determine the significance of proteins. The use of such inhibitors may induce genomic instability in cells, possibly resulting in synthetic lethality in cancer cells with mutated damage repair pathways.

Hypothesis: Many of the proteins recruited to replication forks will be deleted in cancers and remain candidates for biomarkers of sensitivity to treatment

OBJECTIVES

- ❖ To validate the iPOND QMS Data via multiple bioinformatic analytical strategies
  - --via cytoscape
  - --via Cancer BioPortal
- ❖ To develop a prioritization pathway to determine proteins of interest
- ❖ To identify important clusters of interest via Cytoscape
- ❖ To identify which proteins are highly upregulated in all cancers in the TCGA PanCancer Atlas
- ❖ To identify which proteins have high levels of truncation/deletion or amplification mutations

CONCLUSIONS

- ✓ Bioinformatic strategies can be employed to validate experimental data
- ✓ We have developed a prioritization pathway to assist in determining a strong proteomic data set
- ✓ We have identified 82 hits that are upregulated at the replication fork in response to ATRi/WEE1i treatment
- ✓ Cytoscape allows us to identify the function of clusters of proteins, while also taking into consideration log2 fold change and statistical significance (p-value)
- ✓ Cancer BioPortal allows us to identify proteins that are highly regulated in all cancers, as well as organizing by mutation type

3. Cytoscape Analysis to Identify Clusters of Interest

A. AW-o-Edu

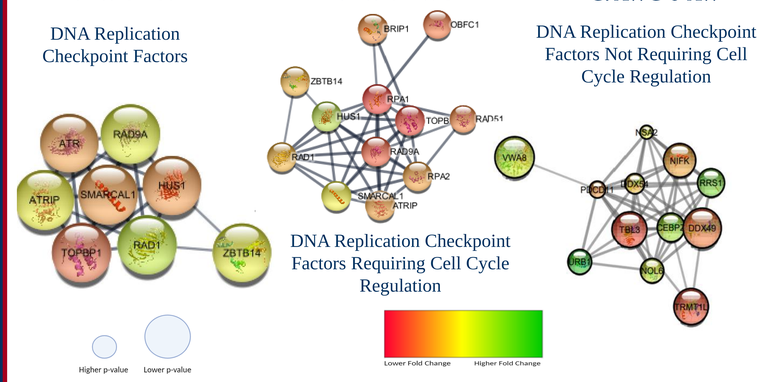
DNA Replication Checkpoint Factors

B. AW-o-AWC

DNA Replication Checkpoint Factors Requiring Cell Cycle Regulation

C. AWC-o-AW

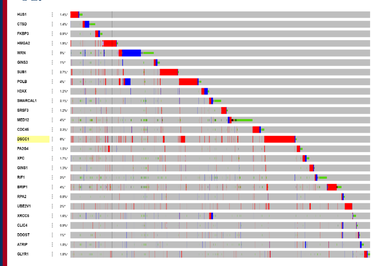
DNA Replication Checkpoint Factors Not Requiring Cell Cycle Regulation



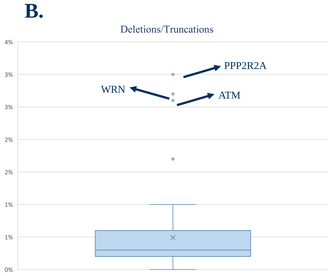
Cytoscape combines protein-protein interactions (STRING) and protein enrichment data (iPOND-QMS). Frequently interacting proteins (drug response factors) are linked together and placed into clusters. Representative images with 0<p-value<2 shown above. Data analyzed via Cytoscape: Cluster in effect of ATRi and WEE1i (**3A**); cluster in the presence of ATRi and WEE1i, with effects in a CDK-dependent manner (**3B**); cluster in the presence of ATRi, WEE1i, and CDKi, with effects in a CDK independent manner (**3C**).

4. Cancer BioPortal Mutation Analysis

A.



B.



Cancer BioPortal allows for the identification of proteins frequently mutated in cancers. Data obtained from the TCGA PanCancer atlas (10,443 samples, 32 cancers). Representative images shown above. Data analyzed via Cancer BioPortal: On the left, an oncoprint with proteins listed and the respective percentages of each type of mutation (4A); on the right, a box and whisker plot of the 82 hits, the respective percentages of the frequency of deletion or truncation mutation, and the inclusion of the factor names of the outliers (4B). Based on the oncoprint, WRN and POLB are candidates because of the high deletion frequency apparent on the left.

Future Directions

- ❑ Experimentally validate the 82 hits by attempting to knockdown the protein
  - --siRNA screen of the 82 proteins, using yH2AX as an indicator of DNA damage (double stranded breaks)
  - --CRISPR Knock Out screen of targets to knockout the gene of interest
  - --We hypothesize that by knocking down these hits, the drug treatment will become more effective
- ❑ Further bioinformatic work to identify if there are proteins that are not highly upregulated in all cancers in the TCGA PanCancer Atlas, but highly upregulated in a specific type of cancer
- ❑ Further bioinformatic wok on Cytoscape, to identify proteins that did not reach the appropriate p-value (statistical significance), but did have the appropriate log2 fold change and interact frequently with one of the 82 target proteins

Thank you to the Brown Lab, PURM Program, CURF, our collaborators, and all funding sources that made this research possible!