Time-Dependent PARP-7 Inhibition Allows for Enhanced Nucleic-Acid Sensing within Breast Cancer

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Background

- Key feature of triple-negative Breast Cancer cells is high chromosomal instability
- Upregulation of PARP-7 by cancer cells is a mechanism that allows them to evade immune sensing of nucleic acids and suppress the Type I IFN response[2].
- RBN-2397, a PARP-7 Inhibitor, is shown to restore immune detection within cancer cells.

Figure 1.1:

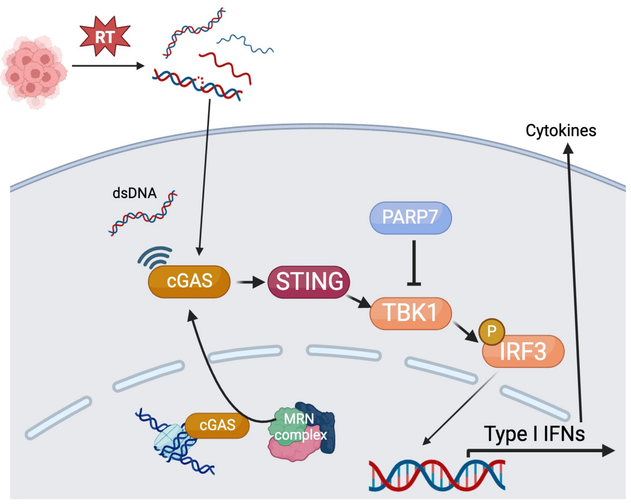


Figure 1.1: Illustration of PARP7 inhibition via MARylation of the nucleic acid sensing cGAS/STING signaling pathway within cells, activated in the presence of cytosolic DNA. The specific protein MARylation is still unknown, but it is hypothesized to be TBK1. Figure adapted from Goddard et al., 2024, *J. Mol. Bio.*[1]

Figure 1.2:

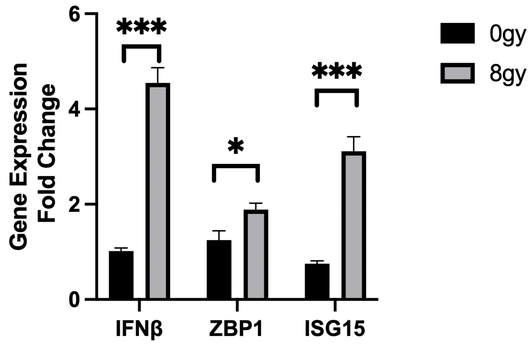


Figure 1.2 Irradiation of 2250L cells shown to induce the expression of interferon stimulated genes. Here, three immunostimulatory genes, IFNb, ZBP1, and ISG15, showed a significantly higher immunogenic response when receiving 8 grays of irradiation as compared to the cells that received 0 grays of radiation, confirming the presence of this innate nucleic acid-sensing immunogenic response.

Research Focus

How can the duration of the drug-target supernatant interaction affect the resulting magnitude of immunogenic signaling through cytosolic nucleic acid detection?

Hypothesis: The greatest immunogenic response will be detected in the RBN-2397 treated cells 24 hours post-transfection. The prolonged interaction between the cell-target and inhibitor supernatant post-transfection will allow for the greatest cytokine induction relative to cell population containing cytosolic DNA, and before nucleic acid degradation.

Quantified by gene expression fold change of immunostimulatory genes IFNβ, a downstream cytokine produced by the cGAS/STING Pathway, and TNFα, a cell death-mediating cytokine.

Methodology

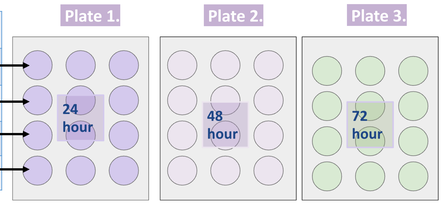
Protocol

1. 2250L cells plated at 100k cells/mL media in a 12-well plate, allowed to adhere overnight.
2. Cells first treated with 50µM of RBN-2397 or DMSO.
3. ISD90/DMSO was transfected 1 hour post treatment using the Lipofectamine 3000 Protocol (Thermo Fisher Scientific).
4. RNA was collected from each well at either 6, 24, or 48-hours, and stored at -80°C.
5. Following RNA isolation, cDNA was made using 1ug of RNA. RT-qPCR was performed using SYBR Green reagents to measure gene expression of target and control genes.

Layout: Experimental Treatments

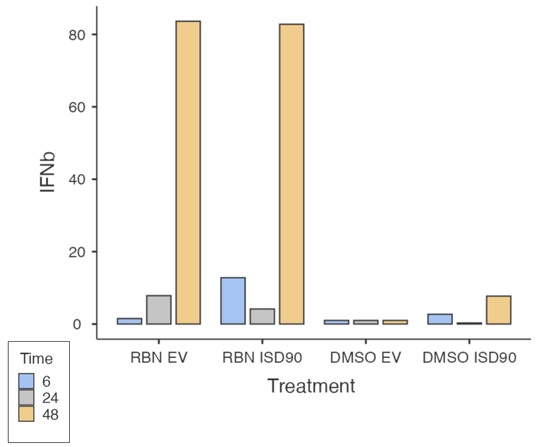
Three 12-well plates used, each row containing one of the following parameters:

Row	Drug treatment & Transfection
1	RBN-2397, ISD90 Transfection
2	RBN-2397, EV Transfection
3	DMSO, ISD90 Transfection
4	DMSO, EV Transfection



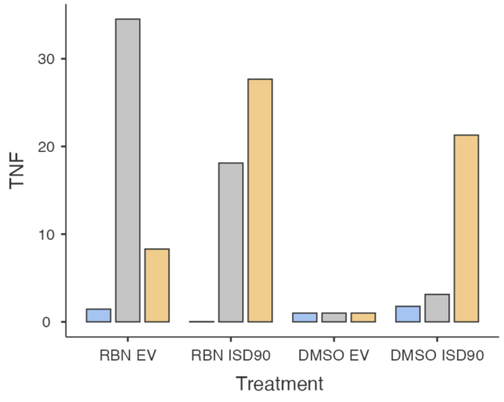
Results

Figure 2.1 **Interferon-beta (IFNβ)**



Greater expression of IFN-β in 2250L cells treated with RBN-2397 compared to DMSO. Within each treatment, a generally positive correlation can be seen between time spent post-transfection and resulting IFN-b fold change, with the greatest observed fold change presented at the 48-hour time point for all four treatments, suggesting an increased induction of an immune response as time passes.

Figure 2.2 **Tumor Necrosis Factor Alpha (TNFα)**



Generally, the data appears to follow a positive trend between time post-transfection and resulting TNFα expression fold change, showing the greatest induction at the 48-hour time point.

Greatest fold change seen in 2250L cells treated with RBN-2397 and no DNA transfection at 24 hours, with the second greatest expression in RBN-2397/ISD90 transfection post 48 hours.

Conclusion: Although a trend in the data suggests a positive correlation between time and magnitude of immunogenic signaling, further experimental inquiry must be done for a more complete understanding of time-dependent nucleic acid sensing in the presence of RBN-2397. Future directions of this can be taken to an *in-vivo* mice model, or more extensive assays to measure magnitude of tumor cell death, and/or protein interactions versus potency of immunogenic response.

Acknowledgements

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References

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