The Mechanism of IB-DNQ-induced Cell Death in NQO1 Positive *BRCA2*-mutant Breast Cancer Cells

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Abstract

BRCA1 and *BRCA2* are tumor suppressor genes that are involved in the processes of DNA repair and gene transcription. When mutated, *BRCA1/2* can lead to the development of breast cancer and are the cause of 5-10% of all breast cancer cases. Unfortunately, current treatments for *BRCA1/2* mutant cancers are not always successful and cause off-target effects in normal tissues. Previous work has shown that the expression of NAD(P)H:quinone oxidoreductase–1 (NQO1) is higher in breast cancer tissues and cell lines than normal tissues. In the presence of certain quinones, such as isobutyl-deoxynybquinone (IB-DNQ), NQO1 performs a two-electron oxidoreduction resulting in futile redox cycling and reactive oxygen species (ROS) generation. We hypothesize that treatment of NQO1+ *BRCA2*-mutant breast cancer cells with IB-DNQ will cause DNA damage and activation of the repair enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Co-treatment of IB-DNQ with PARP-1 inhibitors, ABT-888 or Olaparib (OLA), will therefore show greater toxicity in *BRCA2*-mutant cells due to their inability to repair DNA damage. To test this hypothesis, the *BRCA2*-mutant breast cancer cell line HCC1428 was treated with various doses and times of IB-DNQ with or without the NQO1 inhibitor dicoumarol or co-treated with PARP inhibitors and assessed for viability. To determine whether the NQO1-mediated metabolism of IB-DNQ caused DNA damage, western blot analysis was performed and cell lysates probed with antibodies to phosphorylated H2AX and p53. Results from these experiments suggest that IB-DNQ-induced toxicity in *BRCA2*-mutant cells is NQO1-dependent and causes DNA damage. Future studies will continues to examine the mechanism of IB-DNQ-induced cell death in *BRCA2*-mutant breast cancers.

β-Lapachone (β-Lap) Futile Redox Cycling

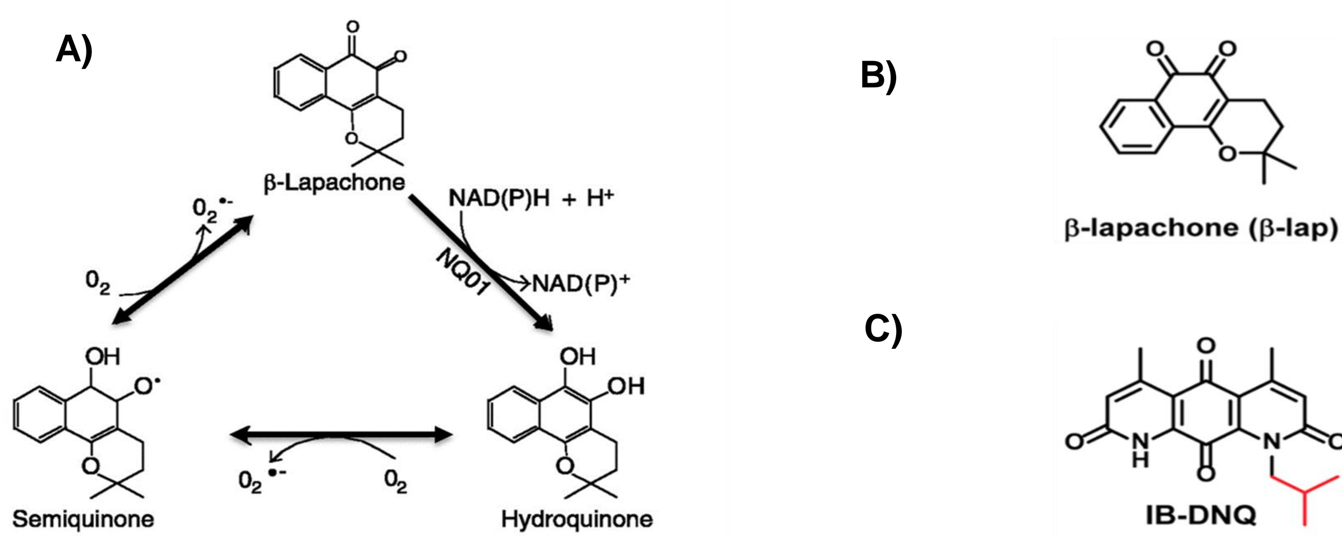

Figure 1. The NQO1-mediated futile cycle of β-lapachone. (A) The parent quinone is reduced to the hydroquinone form. The hydroquinone is further reduced to the semiquinone form, which can cycle back to the parent compound generating reactive oxygen species (ROS). The structure of **(B)** β-lapachone and **(C)** IB-DNQ.

Methods

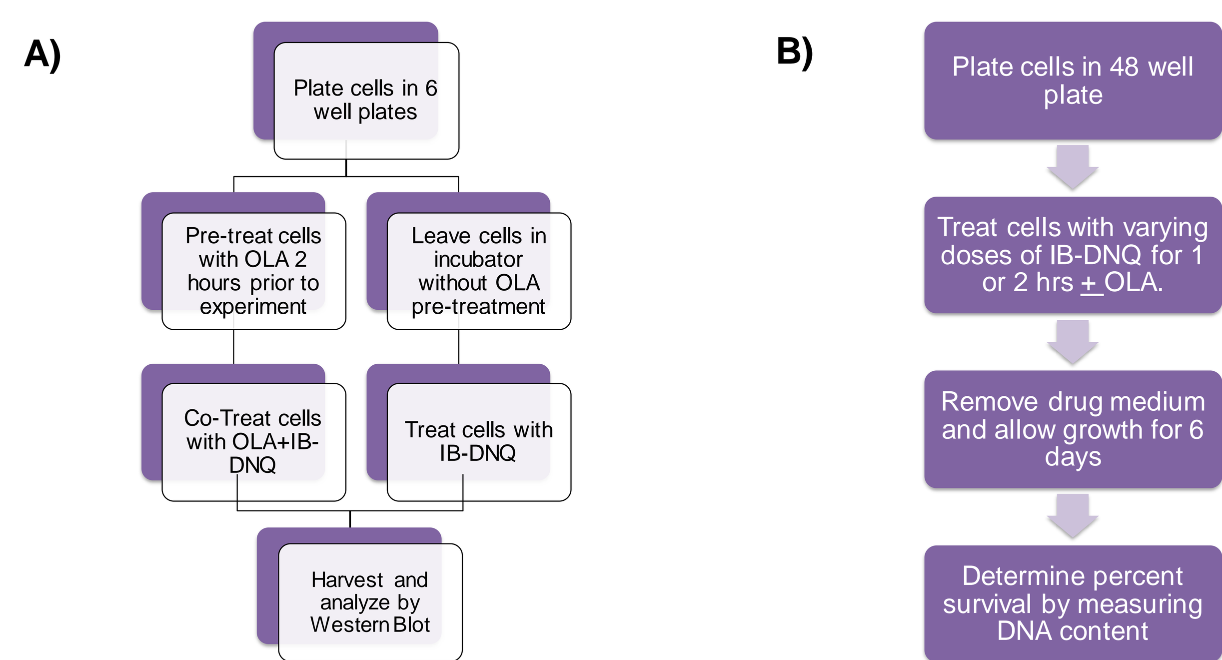

Figure 2. Experimental methods. (A) The *BRCA2*-mutant breast cancer cell line HCC1428 was plated in a 6 well plate and pre-treated or not with the PARP inhibitor, Olaparib (OLA, 15 uM), 2 hours prior to experimental assay. Cells were then co-treated or not with OLA ± IB-DNQ for varying times. Following the final time point, the cells were harvested and analyzed via Western Blot analyses. **(B)** HCC1428 cells were plated in a 48 well plate and treated with varying doses of IB-DNQ over a 1 or 2 hour time frame. After the given time frame, drug medium was removed and cells were left to grow for 6 days. Percent survival was determined via DNA content analysis.

IB-DNQ Toxicity in *BRCA2*-mutant Cells is NQO1-Dependent

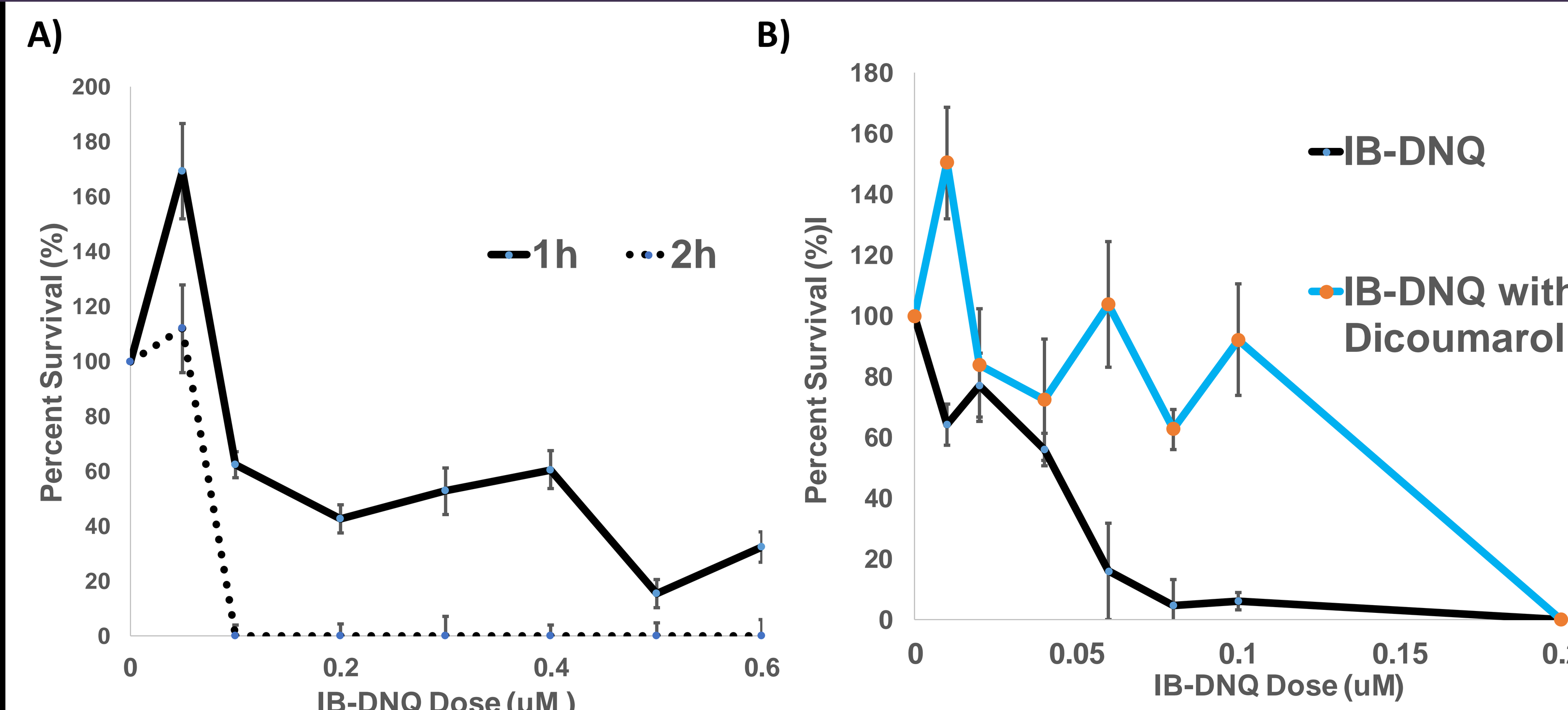

Figure 3. *BRCA2*-mutant cells treated with IB-DNQ show time- and dose-dependent toxicity. (A) HCC1428 cells were treated with increasing doses of IB-DNQ. Drug was left on for 1 h or 2 h, after which time drug medium was removed. Cells were allowed to grow for 6 days, then harvested and analyzed for cell viability. **(B) The NQO1 inhibitor dicoumarol protects cells from IB-DNQ-induced cell death.** HCC1428 cells were treated with increasing doses of IB-DNQ and co-treated or not with dicoumarol. Drug was left on cells for 2 hours and then removed. Cells were allowed to grow for 6 days, then harvested and analyzed for cell viability

*BRCA2-m*utant Cells Show Increased Toxicity when Co-Treated with a PARP Inhibitor

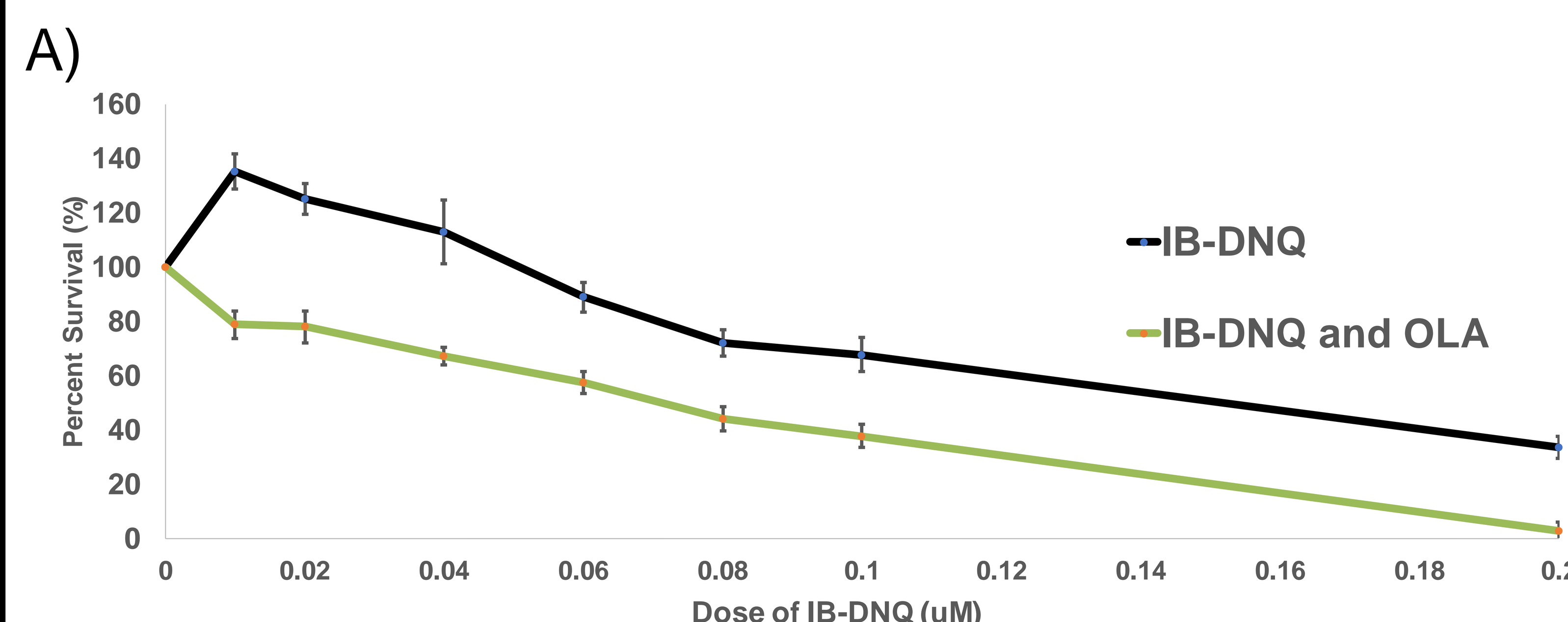

Figure 4. *BRCA2*-mutant cells co-treated with IB-DNQ and PARP Inhibitors show a dose-dependent decrease in cell viability. (A) HCC1428 cells were either pre-treated with Olaparib for 2 hours or not. Cells were then treated with increasing doses of IB-DNQ and Olaparib. Drug media was removed, then cells were allowed to grow for 6 days. Cells were harvested and then analyzed for cell viability. **(B)** HCC1428 cells were pre-treated with ABT888 for 2 hours or not. Cells were then treated with increasing doses of IB-DNQ and ABT888. Once drug medium was removed, the cells were allowed to grow for 6 days and then harvested for cell viability.

IB-DNQ-mediated PARP-1 Activation is Prevented with PARP Inhibitors

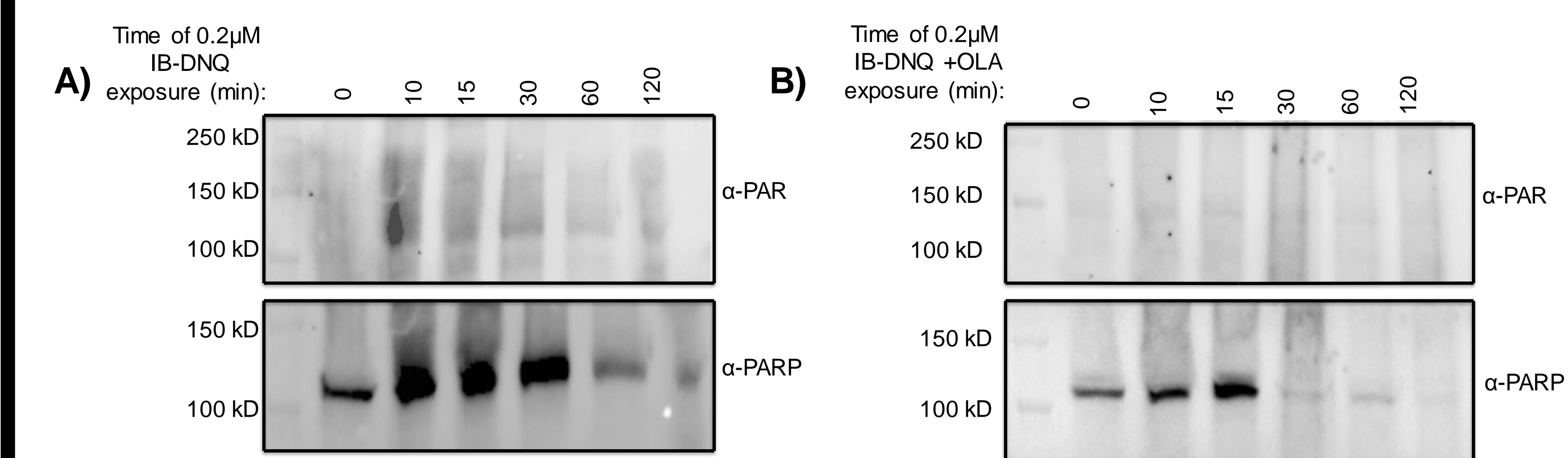

Figure 5. Olaparib prevents IB-DNQ-mediated poly(ADP-ribose) (PAR) polymer formation. (A) HCC1428 cells were plated and treated with 0.2 μM IB-DNQ for 10', 15', 30', 60' or 120 minutes. **(B)** HCC1428 cells were pre-treated with OLA for 2 hours then co-treated with 0.2 μM IB-DNQ for 10', 15', 30', 60' or 120 minutes. Whole cell lysates were harvested at the indicated time points and Western Blot analysis was performed to measure PARP-1 activation by measuring PAR expression levels. Total protein levels were determined using PARP-1 as a loading control.

Working Hypothesis

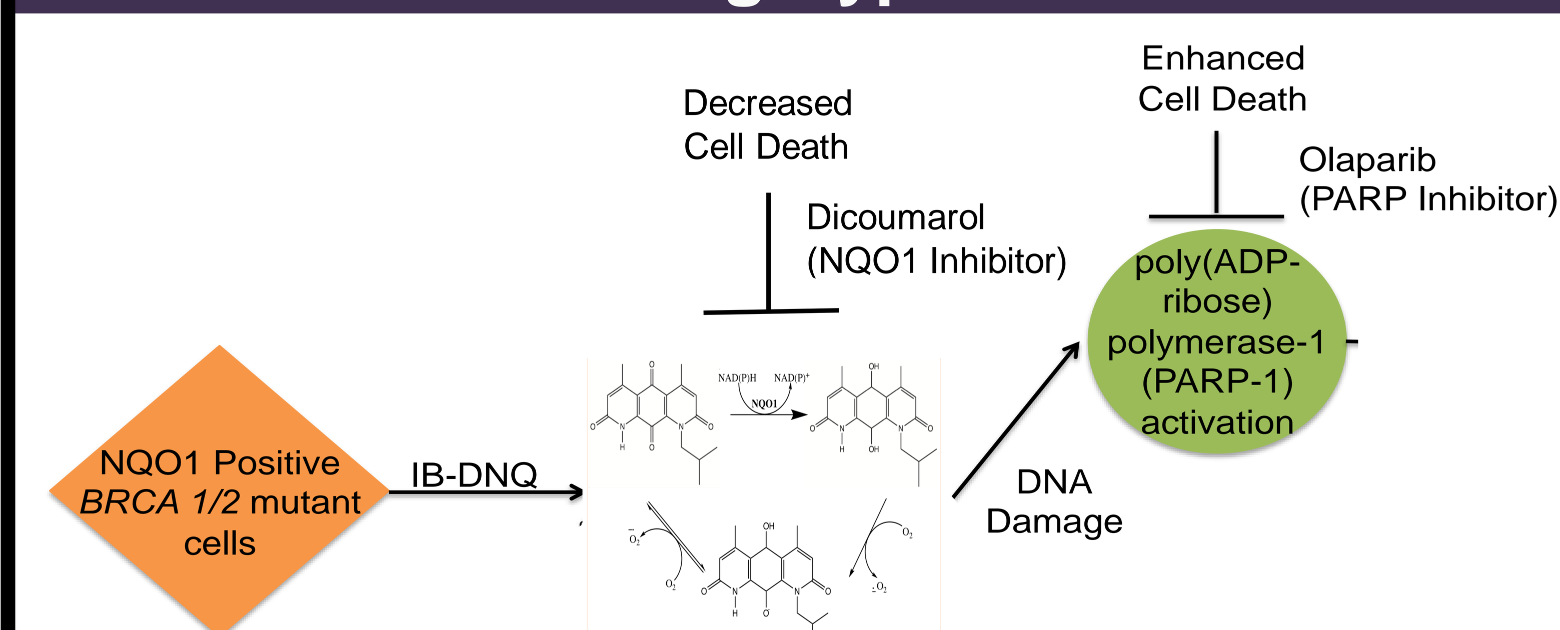

Figure 6. Working Hypothesis. NQO1 is over-expressed in *BRCA 1/2*-mutant cells. When treated with IB-DNQ, NQO1-mediated metabolism of IB-DNQ generates an unstable hydroquinone, resulting in futile redox cycling and dramatic reactive oxygen species (ROS) generation. Generation of ROS leads to DNA damage, thus activating PARP-1. When *BRCA 1/2* -mutant cells are treated with PARP-inhibitor, the DNA damage cannot be repaired, thus resulting in enhanced cell death. When *BRCA1/2* are treated with NQO1 inhibitor Dicoumarol, the futile cycle is prevented, decreasing cell death.

Future Directions

- To determine the sub-lethal doses of IB-DNQ in *BRCA2*-mutant breast cancer cells
- To examine the effectiveness of other PARP inhibitors (Velaparib and Rucaparib) in potentiating IB-DNQ-induced cell death
- To determine the dose- and time-dependence of IB-DNQ-induced DNA damage

Acknowledgements

I would like to thank High Point University as well as the Undergraduate Research and Creative Works Program for providing me with this amazing research opportunity. I would also like to thank my research partners Kristen Brokaw, Lindsey Palmquist, and Kate Hutchinson for all their help in the lab. Many thanks to Dr. Melissa Srougi as well for the opportunity to work in her lab for the past year.