Inhibiting PARP1 Splicing along with Inducing DNA Damage as Potential Breast Cancer Therapy

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Abstract: Triple negative breast cancer is a deadly cancer and once it has metastasized it is deemed incurable. The need for an effective therapy is rising, and recent therapies include targeting the DNA damage response pathway. PARP1 is one of the first responders to DNA damage and has been targeted for inhibition along with the stimulation of DNA damage as a treatment for breast cancer. However, such treatments lack in specificity, and only target one or two domains of the PARP1 protein, whereas PARP1 has other functions pertaining to multiple cancer hallmarks such as promoting angiogenesis, metastasis, inflammation, life cycle regulation, and regulation of tumorigenic genes. In this project, we hypothesize that by inhibiting the PARP1 protein production, we will be able to effectively inhibit all cancer hallmarks that are facilitated by PARP1, and we achieve this by inhibiting the splicing of PARP1. Splicing is the removal of intervening sequences (introns) in the pre-mRNA and the joining of the expressed sequences (exons). For PARP1, we blocked intron 22 splicing by introducing an Antisense Morpholino Oligonucleotide (AMO) that blocks the binding of the spliceosome. The results obtained demonstrate that 50uM PARP1 AMO inhibits PARP1 splicing >88%, as well as inhibits protein production. Additionally, PARP1 AMO lead to a loss in cell proliferation and a loss in DNA damage repair.

Introduction:

- PARP1 is a DNA damage repair protein
- Current Cancer therapies block PARP1 through its catalytic domain
- Available PARP1 inhibitors lack specificity and allow other PARP1 domains to function normally
- Other PARP1 domains can contribute to other cancer hallmarks such as transcription regulation, inflammation and Angiogenesis and metastasis
- This project will block PARP1 splicing (with an AMO) in order to obliterate all functions of PARP1

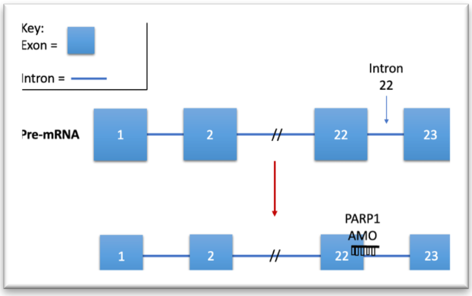
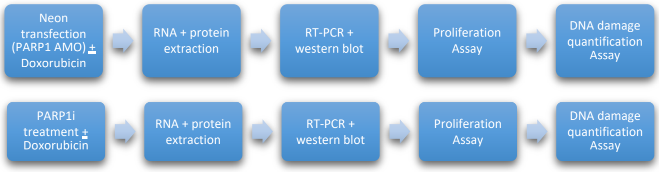


Figure 1: PARP1 AMO, which is a modified RNA, will bind at the junction of exon22-intron 22. This will prevent the splisosome from binding and slicing the mRNA properly.

Hypothesis:

- Inhibiting PARP1 splicing inhibits PARP1 protein synthesis, and all functions related to PARP1.
- PARP1 AMO is more effective and specific than the known PARP inhibitors.
- Coupling PARP1 inhibition to a DNA damaging agent such as doxorubicin would lead to a higher rates of cancer cell death.

Methods:



Results:

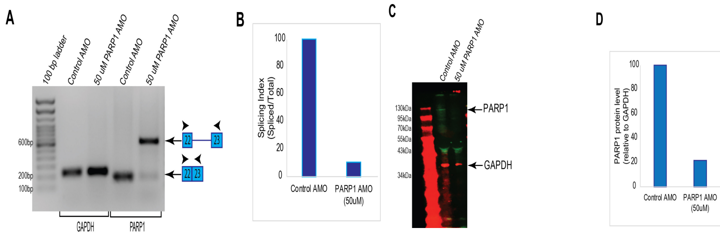


*Figure 2: **A-B:** PCR samples of the PARP1 exon 22- exon 23 region were run on an electrophoresis agarose gel. **C-D:** Proteins were extracted and ran on an SDS gel, and stained for PARP1, GAPDH (loading control). 50uM PARP1 AMO shows high inhibition of PARP1 mRNA splicing and PARP1 protein production. 50uM PARP1 AMO causes a 88.3% splicing inhibition of PARP1 mRNA and a 79.1% decrease in protein production*

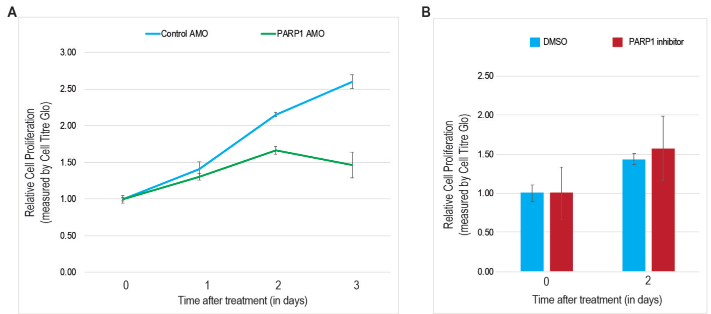


***Figure 3**: A cell proliferation assay was conducted for **A:** AMO-transfected cells and **B:** PARP1i-treated cells. PARP1 AMO alone causes an effect on proliferation, while PARP1i does not.*

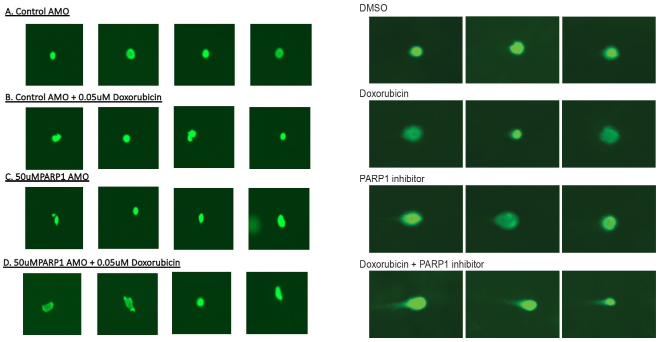


***Figure 4:** A comet assay was conducted for cells transfected with **A:** PARP1 AMO ± Doxorubicin treatment, and **B:** PARP1i ± Doxorubicin treatment. The combination of PARP1 AMO and doxorubicin causes DNA damage in 9/10 cells, while PARP1i + Doxorubicin treatment causes DNA damage in 9/12 cells.*

Conclusions:

- PARP1 AMO does inhibits PARP1 splicing, and protein production
- PARP1 AMO causes a decreasing effect on cell proliferation
- PARP1 AMO inhibits DNA damage repair

Future Work:

- Repeat of some experiments that need statistical significance
- Further functions of PARP1 need to be studied under the influence of PARP1 AMO or PARP1i, such as:
  - a) Upregulation of Inflammation
  - b) Angiogenesis
  - c) Regulation of pro-tumorigenic genes
- Investigating the role of FUS in regulating PARP1 expression

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