Identifying Drug Targets to Radio-sensitize Medulloblastoma

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INTRODUCTION

Fanconi Anemia (FA) is an inherited condition characterized by bone marrow failure and predisposition to malignancy, including medulloblastoma brain tumors. FA occurs due to genetic mutations in the FA/BRCA DNA damage repair pathway which leads to genomic instability and increased toxicity to cancer treatments like radiation. Medulloblastoma (MB) (Fig. 1) is the most common pediatric brain tumor. The MB subtype commonly found in FA patients contain mutations in the Sonic Hedgehog (SHH) pathway leading to its constitutive activation. SHH signaling (Fig. 2) is necessary for embryonic development, and aberrant activation can lead to tumor development. Treatment of SHH-MB tumors includes surgery, chemotherapy and radiation. Since FA patients experience high toxicity to radiation, and radiation induced neurotoxicity is an undesirable side effect of MB treatment in all children, we aim to decrease the effective radiation needed to treat MB. PARP (poly adp-ribose polymerase) (Fig. 3) is a DNA damage repair protein involved in base excision repair. We aimed to investigate whether inhibiting PARP and the SHH pathway can sensitize MB cells to radiation, with the hope of reducing the amount of radiation needed to induce tumor cell death.

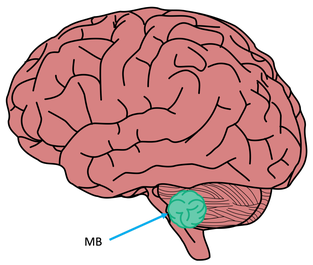

Figure 1: Location of MB

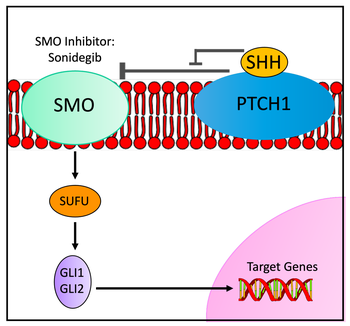

Figure 2: SHH Pathway

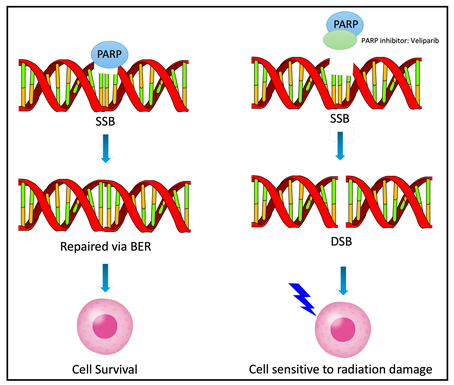

Figure 3: PARP Pathway

METHODOLOGY

Veliparib, a PARP inhibitor, and Sonidegib, a SMO inhibitor were chosen as pre-radiation treatment drugs. Endogenous expression of PARP and SHH intermediates was assessed via western blot and RT-qPCR in both cell lines. In order to identify the optimum drug dose for radiation pre-treatment (IC50), we completed dose response curves using MTT experiments in two human MB cell lines, DAOY and ONS-76. MTT is a colorimetric assay measuring metabolic activity. Drug doses ranged from 0.01-100 μM. Using absorbance measurements from MTT, we measured cell viability over 96 hours in treated cells compared to control.

RESULTS

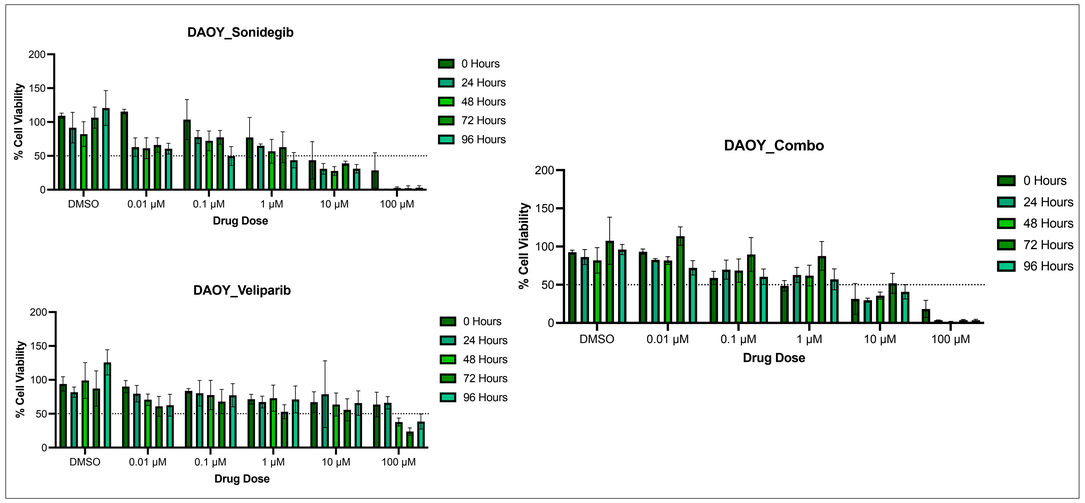

Figure 4: DAOY Single Agent and Combination Treatment Dose Response

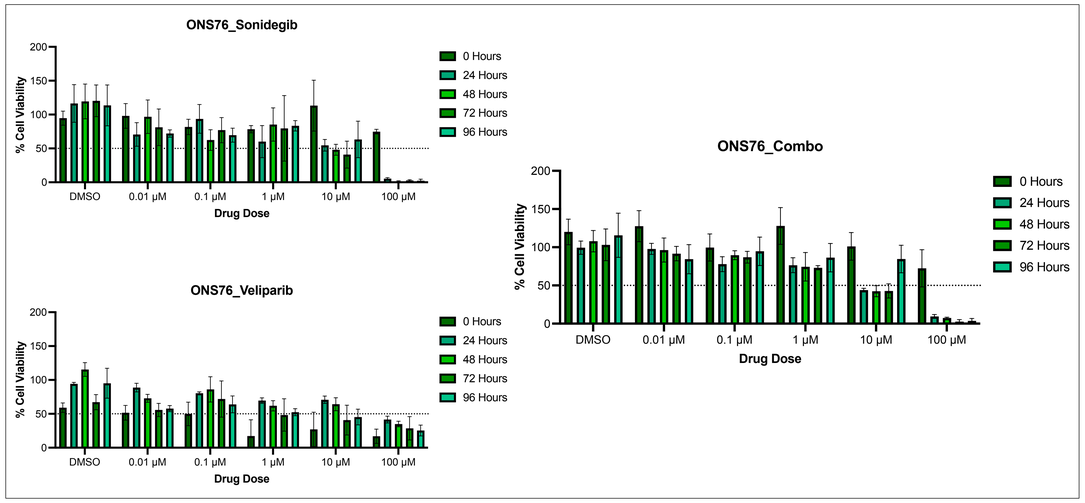

Figure 5: ONS-76 Single Agent and Combination Treatment Dose Response

CONCLUSION

In selecting an optimum dose for drug pre-treatment, we aimed to find the lowest effective dose of drug that reduced cell viability by half (IC50). Using MTT to assess cell viability in both cell lines, we determined 1 μM of Sonidegib and Veliparib is optimum. We will use this dose of drug in future experiments.

FUTURE DIRECTIONS

- Complete radiation dose response experiments to establish baseline responsiveness in DAOY and ONS-76 cells
- Proof of concept experiments measuring RNA and protein expression of PARP and SHH pathway intermediates in response to drugs
- Pre-treat the cells with 1 μM of Sonidegib (PARP inhibitor) and Veliparib (SMO inhibitor) before exposing them to radiation
- Examine radio-sensitization *in vivo* using mouse models

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