Chk1 target validation in recurrent mutant p53 pediatric tumors

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INTRODUCTION

Based on genomic data from the Pediatric Precision Genomics Program at Riley Hospital as well as published studies, many recurrent pediatric solid tumors express mutant forms of the tumor suppressor protein p53. p53's central role in cell cycle arrest and apoptotic pathways has been well studied.

Glioblastoma multiforme (GBM) and Ewing's sarcoma are two cancers with low 5-year survival rates in recurrent pediatric populations. GBM is the most aggressive type of brain tumor, with survival rates that range from 15-30% in pediatrics. Ewing's is a rare cancer of the bone and the soft tissue around the bones with a 70% survival rate for localized tumors and a 30% survival rate for metastatic tumors.

In our laboratory, *in vitro* and *in vivo* studies in mutant p53 GBM and Ewing's sarcoma have demonstrated that the pharmacological inhibition of checkpoint kinase 1 (Chk1) significantly stalls tumor growth, especially when combined with standard-of-care (SOC) DNA-damaging agents. Chk1 is a serine-threonine protein kinase in the DNA-damage response pathway involved in cell cycle arrest. Chk1's secondary role is to regulate DNA replication forks.

To understand the underlying mechanisms of Chk1 inhibition in the context of SOC therapy, we used GBM and Ewing's sarcoma cell lines to evaluate drug effects on cell cycle arrest and Chk1 activation. These studies will help define biomarkers of therapeutic response that can be used to optimize Chk1-targeted therapies for pediatric GBM and sarcoma.

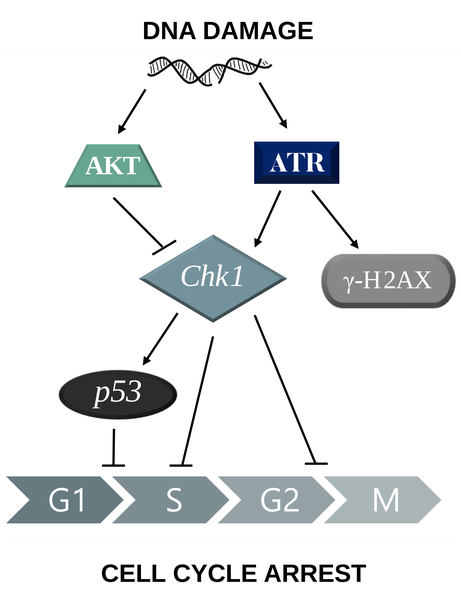

Figure 1. DNA-damage response pathway. Treatment with DNA-damaging chemotherapeutic agents activates ataxia telangiectasia and Rad3-related protein (ATR) and Akt. ATR activates γ-H2AX, a marker for DNA double-stranded breaks. ATR also activates Chk1, which can cause cell cycle arrest in S or G2 phase. Chk1 further activates p53, which arrests cells in G1 phase. Akt can inhibit Chk1 via phosphorylation.

MATERIALS AND METHODS

Cell Line	Type of Tumor
CHLA-10	Primitive neuroectodermal tumor (Ewing Family of Tumors)
SJ-GBM2	Glioblastoma multiforme
TC-71	Ewing's sarcoma

Figure 2. This table describes the cell lines used. Each cell line harbors mutant p53.

Pharmaceutical Compound	Category
CCT245737	Chk1 inhibitor
Irinotecan (SN-38)	Topoisomerase I inhibitor
Lomustine (CCNU)	Alkylating agent

Figure 3. This table describes the pharmaceutical compounds used. CCNU is a standard treatment for GBM. SN-38 is the active metabolite of irinotecan, a SOC for Ewing's sarcoma.

Methods

- Flow cytometry was used to evaluate cell cycle arrest
- Western blot analysis was used to evaluate regulation of Chk1

RESULTS

FLOW CYTOMETRY
GLIOBLASTOMA

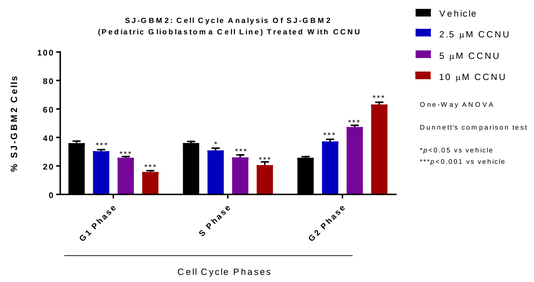

Figure 4. The percentage of SJ-GBM2 cells in specific cell cycle phases after 24 hour treatment with CCNU.

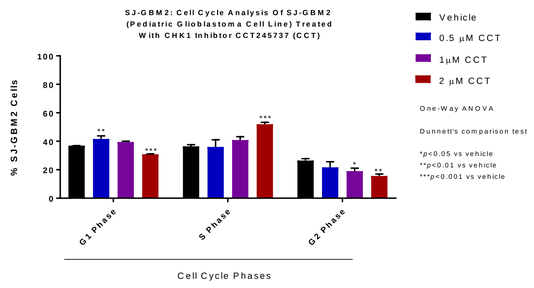

Figure 5. The percentage of SJ-GBM2 cells in specific cell cycle phases after 24 hour treatment with CCT.

Figure 4 shows increased cell cycle arrest in G2 phase after treating GBM cells with CCNU. G2 phase is known to be a cell cycle checkpoint that correlates with Chk1 activation. Figure 5 shows increased cell cycle arrest in S phase after treating GBM cells with CCT. This indicates that treatment with a Chk1 inhibitor causes S phase arrest in GBM cells.

RESULTS CONTINUED

WESTERNS
GLIOBLASTOMA

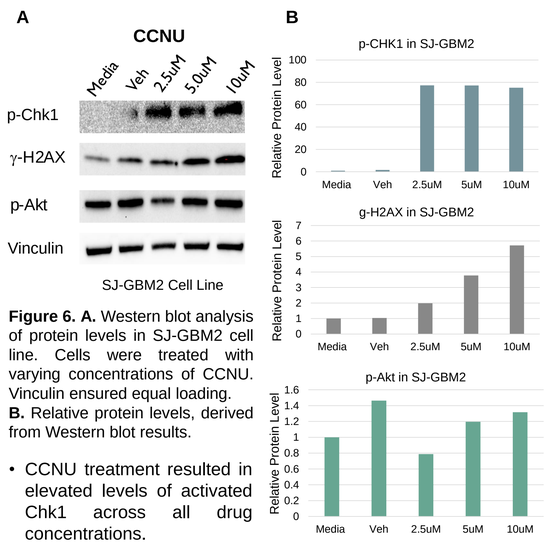

Figure 6. A. Western blot analysis of protein levels in SJ-GBM2 cell line. Cells were treated with varying concentrations of CCNU. Vinculin ensured equal loading.
B. Relative protein levels, derived from Western blot results.

- CCNU treatment resulted in elevated levels of activated Chk1 across all drug concentrations.
- γ-H2AX levels increased linearly with increasing drug concentrations.
- Activated Akt levels were found to be stable across all treatments.

WESTERNS
EWING'S SARCOMA

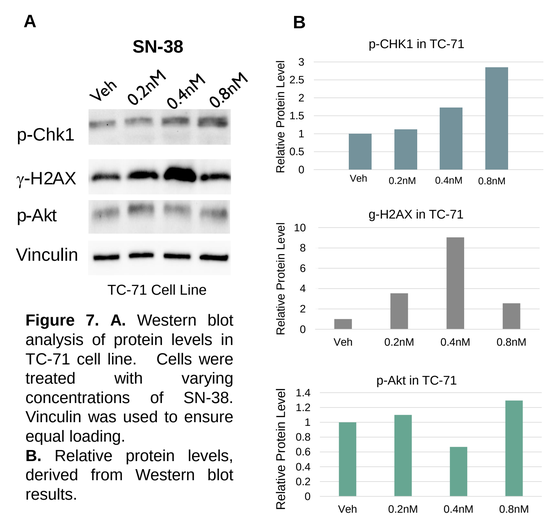

Figure 7. A. Western blot analysis of protein levels in TC-71 cell line. Cells were treated with varying concentrations of SN-38. Vinculin was used to ensure equal loading.
B. Relative protein levels, derived from Western blot results.

- Treatment with SN-38 resulted in elevated levels of activated Chk1.
- Levels of γ-H2AX, a marker for double-stranded DNA breaks, increased with increasing drug concentration, with the exception of the highest dose. This may be because the intense DNA damage caused the cells to die or repair their DNA.
- Activated Akt levels were found to be stable across all treatments.

RESULTS CONTINUED

FLOW CYTOMETRY
EWING'S SARCOMA

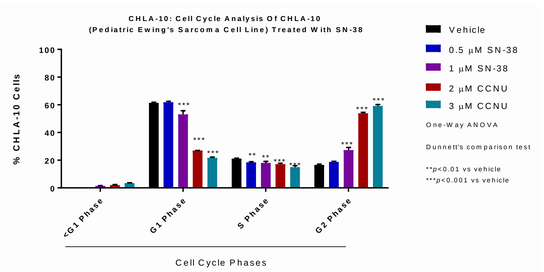

Figure 8. The percentage of CHLA-10 cells in specific cell cycle phases after 24 hour treatment with SN-38.

Figure 8 shows increased cell cycle arrest in G2 phase after treating Ewing's tumor cells with SN-38. G2 phase is known to be a cell cycle checkpoint that correlates with Chk1 activation.

CONCLUSIONS

- Via Western analysis, Chk1 activation was confirmed in both GBM and Ewing's when treated with standard-of-care DNA-damaging agents.
- Flow cytometry showed that inhibition of Chk1 results in cell cycle arrest in S phase.
- Flow cytometry results also showed that DNA-damaging agents cause cell cycle arrest in G2 phase.

FUTURE DIRECTIONS

- Validate Chk1 inhibition by evaluating downstream targets of Chk1, such as Cdc25
- Evaluate cell death mechanisms
- Conduct pharmacodynamic *in vivo* studies to validate Chk1 activation versus inhibition
- Evaluate combination therapies with Akt inhibitors and Chk1 inhibitors that could block the DNA-damage response pathway

REFERENCE

DNA Image in Figure 1 from:
Sebald, Angelika. "Physical Principles (Radiotherapy)." Edited by David A. Mitchell, *Maxfacts*, British Association of Oral and Maxillofacial Surgeons , 23 Aug. 2017, maxfacts.uk/treatment/radiotherapy/principles/detailed.
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