ROMIDEPSIN THERAPY RESULTS IN P53 MEDIATED APOPTOSIS IN HEPATOBLASTOMA

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Background: Relapsed and treatment refractory hepatoblastoma (HB) has a survival rate less than 50% due to limited treatment options. We have previously shown that HB and treatment refractory HB has upregulation of class I histone deacetylase (HDAC) genes (HDAC1-4) through microarray. The highest consistent upregulation noted was with HDAC 2 and 3. Given this upregulation, we tested established and treatment refractory HB cell lines with HDAC 2/3 inhibitor, Romidepsin. We sought to characterize Romidepsin efficacy and mechanism of action.

Materials/Methods: Romidepsin was tested with cytotoxic assays (MTT). Tumor derived cell lines were treated with Romidepsin and evaluated for gene expression of p53 pathway via qRT-PCR. Immunoblotting assays were used to assess changes in expression of Acetyl-p53 at time points of 0, 4, 8, and 24 hours after 0.01 □M concentrated Romidepsin treatment. Evaluation of the specific cell death pathway involved was performed via cell viability assays using 0.01 □M concentration of Romidepsin in combination with cell death reversal agents.

Results: Romidepsin (IC50 of 0.001-0.02 □M) showed strong in vitro effects for viability. HB cell lines treated with HDAC inhibitor had increased p21 and PUMA expression on RT-PCR, most notably at the 24-hour mark ($p = < 0.05$). Acetyl p-53 was noted to increase in HUH-6 and Hep G2 throughout the 24 hours of treatment on western blots, while Hep T1 was noted to have no changes in expression. All cell lines showed the highest level of cell death reversal with ZVAD in cell viability assay after Romidepsin treatment.

Conclusions: Romidepsin demonstrates strong in vitro efficacy and suggest HDAC 2/3 inhibition may act through p53 mediated mechanism, notably acetylated p53. Cell death via apoptosis is further supported by cell death reversal with ZVAD/Romidepsin treatment in HB cell lines.

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