HISTONE DEACETYLASE INHIBITORS (HDACI) TARGET NEUROBLASTOMA TUMOR STEM CELL ENRICHED SIDE POPULATION

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Objective: Current evidence suggests that tumor stem cells (TSC) evade conventional chemotherapeutics, resulting in tumor relapse. Patients with high-risk neuroblastoma (NB) retain poor prognosis even following treatment with current chemotherapeutics. HDACi are undergoing phase I trials in NB, however their ability to target NB TSCs is not known.

Hypothesis: HDACi treatment is able to target the highly tumorigenic NB TSC population.

Methods: Hoechst 33342 exclusion identifies a side population (SP) of NB cells that have been shown to be enriched in highly tumorigenic TSCs. N-type NB cell lines SK-N-BE (2) and SH-SY5Y, and NT2/D1 teratocarcinoma cell lines were examined. Tetartocarcinoma, a germ cell tumor line was used as a model of TSC. Changes in SP were analyzed following a 48 hr LD50 treatment with chemotherapeutic cisplatin (10 uM) and HDACi trichostatin A (TSA; 100nM) and MS-275 (1.5 uM) assayed by methylcellulose assay. OCT-4 and SOX2, stem cell markers previously shown to be overexpressed by TSCs, were analyzed by western blot and flow cytometry.

Results: HDACi TSA decreased SP in NB SH-SY5Y and SK-N-BE (2) by 55.4 and 90.6% respectively ($p = 0.0286$ and $p = 0.0095$). MS-275 significantly decreased the percentage of SP cells by 75.1 and 77.89% in NB SH-SY5Y and SK-N-BE (2) and by 92.2% in NT2/D1 ($p = 0.0059$; 0.0180; 0.0081). Cisplatin had no significant effects on SP in all 3 cell lines. At 750 nM MS-275, there were no visible colonies. OCT-4 and SOX2, which have previously shown to be overexpressed in NB TSCs, was significantly downregulated by HDACi MS-275 treatment to 49.6 and 45.78% of control ($p = 0.0003$ and $p = 0.0066$).

Conclusion: Our in-vitro results suggest that HDACi may be a potent anti-NB therapeutic because of its ability to target NB TSCs.

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