**Research Background**

Diffuse Intrinsic Pontine Glioma (DIPG) is a glial tumor occurring primarily in children with extremely poor prognosis. Occurring outside of the pons, surgery following diagnosis is not viable for DIPG patients as the location of the tumor is vital for motor functions. There has yet to be a significant improvement in the survival median rate upon diagnosis outside of the effects of radiation therapy. Though radiation therapy has proven to improve neurological symptoms of DIPG patients, the results of this treatment have proven to be transitory at best. Currently, there is an interest in looking at specific oncogenic targets to find the most efficacious biological agent(s) to decrease tumor cell growth.

Mithramycin is a relatively new anticancer drug reported to be effective against a variety of cancers. It blocks Specificity protein-1 (Sp1) from binding to DNA through its guanine-cytosine (G-C)-specific DNA-binding ability and selectively downregulates an X-linked inhibitor of apoptosis protein (XIAP) levels, therefore increasing levels of apoptosis in cancer cells. SET Domain Bifurcated Histone Lysine Methyltransferase 1 (coded by SETDB1 gene) is an enzyme that reversibly catalyzes methylation in Histone 3 K9 (H3K9). SETDB1 is heavily involved in the downregulation of vital tumor-suppressive genes, adding to its pro-oncogenic nature and is recently found to be overexpressed in DIPGs by our lab. Mithramycin was reported to be a SETDB1 inhibitor by blocking the interaction between Sp1-1 and SETDB1 regulatory sites.

Since oncogenic proteins REST and SETDB1 interacts with each other and are overexpressed in DIPGs, targeting their oncogenic interaction may prove to be beneficial for DIPG patients. Interaction of REST and SETDB1 indicates towards cooperative oncogenic behavior in DIPGs.

Hypothesis

Since oncogenic proteins REST and SET DB1 interacts with each other and are overexpressed in DIPGs, targeting their expression/blocking interaction may prove a novel therapeutic approach. Using mithramycin may result in interfering with SET DB1 and REST oncogenic interaction and inhibit DIPG proliferation.

**Methods**

Cell Culture

TSM base media was prepared by mixing equal volumes of Neurobasal-A and DMEM/F12 mix along with growth factors. Fresh culture media was prepared by adding EGF, FGC, PDGF, Heparin and other growth factors in TSM base medium and used to culture all three cell lines.

Treatments

Mithramycin was dissolved in DMSO at 10mM concentration followed by serial dilutions in complete media. 10 & 100uM Stocks were used to administer treatments in 96 well plates for 120 hours.

MTT Assay

Cells were plated in 96-well plates for an incubation period of 120 hours. For adherent cells, media is suctioned from the plate. For suspension cells, cells are centrifuged at 4 degrees Celsius for 5 minutes before media is sanitized. 50uL of media per well followed by 50uL of MTT solution per well is added to 96-well plates. Cells are incubated at 37 degrees Celsius for 4 hours. Following incubation, 150 uL of MTT solvent is added to each well. Absorbance is read at OD=590nm within 1 hour.

**Summary of Results**

REST and SET DB1 are overexpressed in DIPG tumors and contribute to proliferative potential. Interaction of REST and SET DB1 indicates towards cooperative oncogenic behavior in DIPGs. REST and SET DB1 occupies Caveolin -1 promoter and induce trimethylation of H3K9. Targeting SET DB1 expression using Mithramycin results in significant decrease in cell proliferation in all DIPG cell lines.

Slightly lower toxicity in REST high cell lines indicate REST might be rescuing against loss of SET DB1 expression.

Cell Cycle analysis showed decreased population in G2/M phase for DIPG7 cells while in DIPG7REST, there is significant decrease in cell populations in S and G2/M phase.

**Conclusions**

Mithramycin is able to induce cell death in DIPG cell lines. REST-SET DB1 interaction axis is need to be evaluated for its potential impact on DIPG tumor survival. Detailed mechanism of Mithramycin action is needed to be elucidated.

**References**


