Combinatorial Inhibition of Epigenetic Regulators to Treat Glioblastoma

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Background: Glioblastoma multiforme (GBM) is a deadly primary brain cancer that is diagnosed in 12,000 patients in the US annually with a median survival time of 15 months. Temozolomide is the standard-of-care for GBM; however, many tumors are resistant, necessitating the expansion of therapeutic options. EZH2 and JMJD3 are two proteins responsible for epigenetic regulation of the genome via histone methylation, with EZH2 also affecting non-histone targets. Prior studies showed that inhibition of these proteins decreased cell counts and induced radiosensitivity in GBM. Thus, we investigated combined use of EZH2 inhibitor, EPZ-6438, and JMJD3 inhibitor, GSK-J4, in the treatment of temozolomide-resistant GBM10 cells.

Methods: Non-irradiated cells were treated with both drugs singly or combined, and counted at 24-, 48-, and 72-hour intervals. Irradiated cells were pre-treated with each drug or combination therapy for three days, irradiated, and then counted at 24-, 48-, and 72-hour intervals. Western blot allowed investigation of dsDNA damage biomarker yH2AX, gene-silencing modification H3K27me3, total H3, tumor suppressor p53, EZH2, JMJD3, ySTAT3, and total STAT3 expression in non-irradiated and irradiated cells following drug treatment.

Results: Single EPZ-6438 and GSK-J4 treatment decreased cell count in a dose and time dependent manner. GSK-J4 was more effective than EPZ-6438, and combinatorial treatment was most effective. Western blot revealed that GSK-J4 but not EPZ-6438 treatment followed by radiation increased H3K27me3 expression. EPZ-6438 treatment increased yH2AX expression, but this was not further increased by radiation. Meanwhile, GSK-J4 treatment increased yH2AX, but only after radiation.

Discussion: Decreased cell count following GSK-J4 treatment may be due to increased gene silencing resulting from the inhibition of H3K27 demethylation. Additionally, increased dsDNA breaks observed in EPZ-6438 and GSK-J4 treatments supports their roles in radiosensitizing GBM cells.

Potential Impact: This study highlights the importance of further investigation into GSK-J4 and EPZ-6438 combination therapy in temozolomide-resistant GBM tumors.