Proliferative Inhibition of Glioblastoma Tumor Initiating Cells (GTICs) with Ribonucleotide Reductase Inhibitors
Ritesh Kumar MD; Ian Lorimer
Centre for Cancer Therapeutics, Ottawa Hospital Research Institute

Introduction: Glioblastoma, the most common primary malignant brain tumor, has a median survival of 14 months. Recent studies suggest a non-differentiated population of tumor cells (GTICs) are the key drivers of glioblastoma (1). Premature senescence is the irreversible cessation of proliferation despite continued metabolic activity before the end of the cellular replicative lifespan. It is often the result of DNA damage and is independent of telomere attrition. Induction of premature senescence in cancer cells has been shown to be a promising cancer therapeutic but it has not been well studied in GTICs (2).

Hypothesis: Induction of premature senescence in GTICs by DNA damage can reduce the malignant potential of glioblastoma.

Methods: GTICs were harvested from human patients with glioblastoma (Figure 1). Double stranded DNA (dsDNA) damage was induced in PriGO8A GTICs using X-ray irradiation. Single stranded DNA (ssDNA) damage was induced in PriGO8A GTICs with inhibitors of ribonucleotide reductase Triapine or Hydroxyurea. Cell proliferation was measured by cell counts and senescence was analyzed by the senescence associated beta galactosidase (SA-B-Gal) assay. Cell death was measured by propidium iodide staining.

Results

Figure 2: The signalling pathways that respond to DNA damage and converge on key decision making proteins such as p53 to mediate transient arrest, apoptosis, or cellular senescence. Ionizing radiation causes dsDNA damage whereas Triapine and Hydroxurea cause ssDNA damage by inhibiting ribonucleotide reductase. Ribonucleotide reductase converts ribonucleotides to deoxyribonucleotides and its inhibition leads to a depletion of deoxyribonucleotides resulting in stalling of the replication fork.

Figure 3: PriGO8A GTICs respond to radiation with decreased proliferation. A: Cell counts performed 48 hrs after irradiation demonstrated reduced proliferation in a dose dependent manner. There is no significant difference between total and live number of cells in any of the conditions suggesting radiation does not induce cell death in PriGO8A GTICs. B: SA-B-Gal performed 7 days after irradiation fails to demonstrate senescence as a response to radiation in PriGO8A GTICs.

Figure 4: PriGO8A GTICs respond to Triapine treatment by undergoing cell death and increased senescence. A: Cell counts performed 48 hrs after Triapine treatment show a 3x reduction in proliferation compared to untreated cells. B: SA-B-Gal staining performed 7 days after 10 mM treatment shows 75% positivity compared to 5% in untreated cells. C: Hydroxyurea treatment does not significantly decrease the viability of PriGO8A GTICs.

Figure 5: PriGO8A GTICs respond to Hydroxyurea primarily by undergoing senescence. A: Cell counts performed 48 hrs after treatment show a 3x reduction in proliferation in 10 mM treated cells compared to untreated cells. B: SA-B-Gal performed 7 days after 10 mM treatment shows 75% positivity compared to 5% in untreated cells. C: Hydroxyurea treatment does not significantly decrease the viability of PriGO8A GTICs.

Conclusions: GTICs appear to be more sensitive to ssDNA than dsDNA damage. GTICs may enter a state of quiescence in response to radiation with a temporary inhibition of proliferation. However, GTICs respond to inhibitors of ribonucleotide reductase with permanent cessation of cellular proliferation by undergoing either cell death or premature senescence. Inducers of ribonucleotide reductase appear promising in the treatment for glioblastoma.

Future Directions: Further in vitro characterization of the response of PriGO8A GTICs to Triapine and Hydroxyurea. Assessment of the in vivo response of PriGO8A GTICs to Triapine and Hydroxyurea in a xenograft mouse model.

References: