**BACKGROUND**

- Diffuse midline gliomas (DMGs) are common childhood cancers that originate in the brain stem and constitute about 10-20% of all childhood brain tumors.
- Radiation therapy (RT) is the only effective treatment, which at best extends life, the 5-year survival rate is 0% since the tumors become resistant to RT while repairing the DNA damage arising from RT.
- The cause of radioresistance has not been studied in DMGs until now.

**METHODS**

- A DNA repair shRNA screen was performed to determine the most common genes lost after 21 days, between unirradiated SF8628 cells and those radiated at 60 Gy.
- SF8628 cells were treated with LiCl of the NCI drug panel after RT with 60 Gy and compared to unirradiated cells (60 Gy) treated with the same panel.
- RT-PCR and western blotting was used to determine the effect of single dose of 60 Gy RT on the anti-apoptotic protein, BCL2, on DMGs SF8628, BT245, MAF-002.

**RESULTS**

1. **Integrated genomics analysis identifies B-Cell Lymphoma 2 (BCL2) as a key regulator of DMG cell survival after RT**

   - **A.** Western blot of BCL2 expression in DMG patient tumors and normal pons. **B.** BCL2 expression in DMG cells from cell lines cultured from tumor biopsies (SF8628, BT245, MAF-002) that are subjected to 60 Gy RT compared to RT-sensitized cells by DAPI. **C.** Western blot of BCL2 protein expression in OvarY or 60 Gy RT SF8628 cells. **D.** IHC analysis of BCL2 in BT245 and SF8628-Luc2 xenografts, suggesting that BCL2 is involved.

2. **BCL2 is upregulated after RT and venetoclax is a potent inhibitor of BCL2 activity, growth and proliferation following RT in DMG.**

   - **A.** BCL2 expression in DMG patient tumors and normal pons. **B.** BCL2 expression in DMG cells from cell lines cultured from tumor biopsies (SF8628, BT245, MAF-002) that are subjected to 60 Gy RT compared to RT-sensitized cells by DAPI. **C.** Western blot of BCL2 protein expression in OvarY or 60 Gy RT SF8628 cells. **D.** IHC analysis of BCL2 in BT245 and SF8628-Luc2 xenografts, supporting the role of BCL2.

3. **Venetoclax binds to BCL2 following RT preventing the binding of BIM augmenting mitochondrial ROS and apoptosis in DMG.**

   - **A.** Venetoclax binding to BCL2 in DMG cells following RT. **B.** Venetoclax binding to BCL2 in DMG cells following RT. **C.** Venetoclax binding to BCL2 in DMG cells following RT. **D.** Venetoclax binding to BCL2 in DMG cells following RT. **E.** Venetoclax binding to BCL2 in DMG cells following RT. **F.** Venetoclax binding to BCL2 in DMG cells following RT.

4. **Venetoclax in combination with RT improves survival in orthotopic mouse BT245-Luc2 xenografts by increasing tumor cell apoptosis.**

   - **A.** Representative bioluminescence images of the BT245-Luc2 cells before, after, and during the specified treatments. **B.** Kaplan-Meier survival analysis of BT245-Luc2 cells bearing mice with indicated treatments, vehicle, n=8; VEN, n=7; RT, n=10; RT+VEN, n=8 mice, and median survival in days. **C.** Complete blood count analysis, in mice. **D.** Venetoclax expression in venetoclax treated mice, as measured from the green fluorescence of caspase3/7 staining and measured using hyperfluorescence. **E.** Western blots showing PARP cleavage in venetoclax treated mice with indicated treatments, as measured from the green fluorescence of caspase3/7 staining and measured using hyperfluorescence.

**CONCLUSIONS**

- **B-Cell Lymphoma 2 (BCL2) was identified as a key regulator of tumor growth after RT in DMGs.**
- **RT sensitized DMGs to venetoclax treatment independent of p53 status.**
- **While venetoclax as a monotherapy was not cytotoxic to DMG cells, post-RT venetoclax treatment significantly increased cell death, reduced BCL2-BIM association and augmented mitochondrial ROS leading to increased apoptosis.**
- **Combining venetoclax with RT significantly enhanced the survival of mice with DMG tumors.**
- **Venetoclax, combined with RT, induced the anti-apoptotic function of BCL2 to augment DMG cell survival.**

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