Combined Bcl-2 & BH3 Profiling as a Method to Define Therapeutic Response and Resistance in Grade IV Astrocytomas

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Introduction

Grade IV astrocytomas, formerly known as glioblastoma multiforme (GBM), are the most common primary brain tumors and have the highest mortality. The therapeutic standard for managing this malignancy remains a combination of surgery, chemotherapy, and radiotherapy; however, there is no cure, nor has there been any significant advancement in the clinical approach to GBM since this protocol was established in 2005. This study aims to better understand the molecular and metabolic characteristics of GBM-derived cell lines to better define treatment groups and potentially identify new avenues for therapy.This study utilized ten continuous GBM cell lines and examined the concentrations of Bcl-2 family proteins on mitochondria, then correlating these values to drug suceptibility.

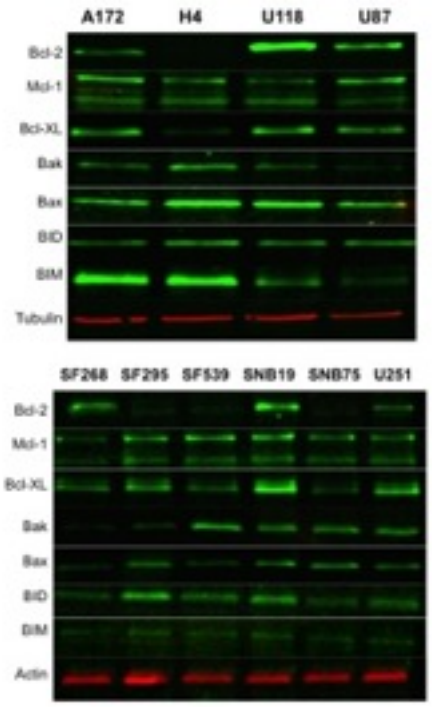

Figure 1: Bcl-2 protein profiling of GBM cell lines. Glioblastoma cell lines were grown to 85% confluency and lysed. Proteins (40ug) were resolved by SDS-PAGE and analyzed by quantitative western blot. (A) ATCC cell panel (B) NCI-60 cell panel.

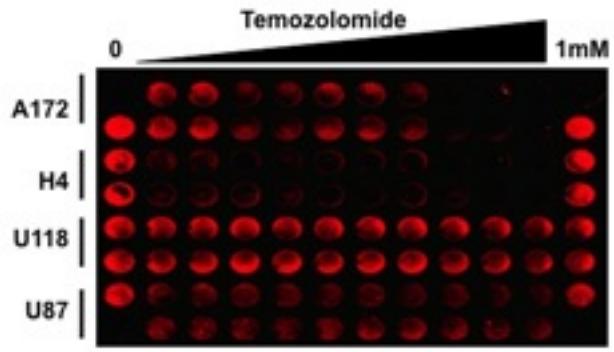

Table 1: TMZ IC_{50} Values for Established GBM Cell Lines

Cell Line	TMZ IC_{50} (mM)
U118	>10 mM*
SNB19	>10 mM*
U251	>10 mM*
U87	0.75 ± 0.11
SF295	0.64 ± 0.08
SF539	0.37 ± 0.03
SF268	0.21 ± 0.10
A172	0.18 ± 0.05
SNB75	0.07 ± 0.02
H4	0.04 ± 0.01

IC_{50} Values for GBM cell lines were determined in a 96-well format on $1.5x10^4$ cells/well using cell-based fluorescence. * The dose curve for this experiment was from 0-10mM due to the solubility of TMZ.

Figure 2: Calculation of TMZ IC50. Glioblastoma cell lines were treated with increasing concentrations of TMZ (0-10mM). After 48 hours cells viability was measured using TO-PRO-3 stain (680nM) (A, representative assay). Cells were plated at a density of 1.5x104 cells/well. IC50 values were calculated using GraphPad® Prism (B).

Methods

Cellular viability in the presence of increasing doses of TMZ was determined for ten continuous GBM cell lines. Concentrations of Bcl-2 family of proteins were obtained via Western blot for each cell line. Measures of Bcl-2 family proteins was then correlated to IC50 values for TMZ.

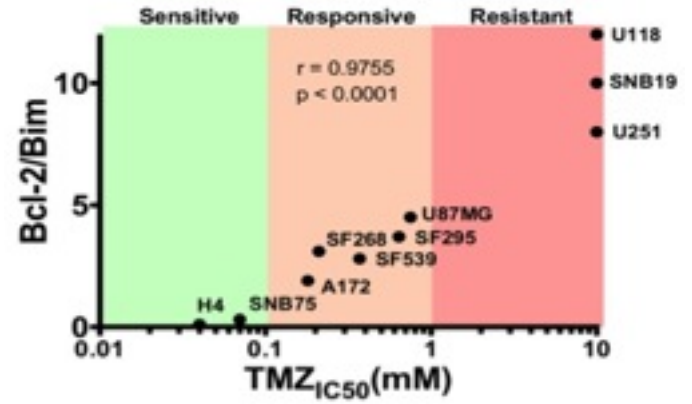

Figure 3: Correlation of Bcl-2/Bim to TMZ Sensitivity. The Bcl-2/Bim ratio was calculated for each of the GBM cell lines and plotted against the corresponding IC50 for TMZ. Spearman correlation test was used to determine the correlation coefficient (r)

Results

Western blot analysis of pro-survival and pro-apoptotic Bcl-2 proteins revealed that Bcl-2 levels corresponded to chemo-sensitivity, while increased levels of Bim promoted chemo-sensitivity in GBM cell lines. The ratio of Bcl-2 and Bim expression was found to be significantly correlated ($p<0.0001$) to TMZ responsiveness ($r = 0.9755$). Induction of TMZ resistance in U87 cell by exposure to a hypoxic environment increased the Bcl-2/Bim ratio.

Conclusions

Herein, we demonstrated that established GBM cell lines have distinct mitochondrial phenotypes that may contribute to chemo-responsiveness.

Specifically, we observed:

1. Bcl-2 levels are high in chemo-resistant cells.
2. Bim concentration is elevated in chemo-sensitive cells.
3. Bcl-2:Bim ratio corresponds to TMZ sensitivity in cells.