Targeting Metabolism in Brain Tumors

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Introduction

According to Warburg, irreversible cellular respiratory damage causes cancer cells to depend entirely upon aerobic glycolysis. We propose that targeting aerobic metabolism will be an effective strategy for treating gliomas.

Methods

Glioblastoma (GBM) cell lines U87, U251, LN229, and CCF 247; a primary line obtained from our brain tumor bank, were used for in vitro studies. Normal human astrocytes (HA) obtained from normal brain from epilepsy surgery patients, were also evaluated in vitro. Cells were treated with 3-Bromopyruvate (3-BrPA), a lactate/pyruvate analog which selectively depletes ATP and inhibits Hexokinase-2 dependent aerobic metabolism. We evaluated the effects of 3-BrPA treatment with use of TUNEL (DNA Flow Cytrometric Analysis Kit, Roche Diagnostic Corp) and flow cytometry (Becton Dickinson FACScan, San Jose, Ca.). Caspase activity was measured using the Apo-ONE Homogeneous Caspase-3 assay (Promega, Madison, WI).
Institutional animal facility approval was obtained for in vivo studies. Tumor cells were implanted in the flank of athymic nude rats. Ten days later, rats were treated with either 3-BrPA or PBS by direct tumor injection every seventy two hours for three weeks. Tumors were measured every forty eight hours and tumor volume was calculated.

Discussion

The initial question in this project was to determine if 3-BrPA was able to induce apoptosis (programmable cell death). To answer that question, we used a series of commercial Glioblastoma cell lines; U87, LN229, and U251 and the primary GBM cell line CCF 247.
We compared that with a white matter cell line obtained from normal brain from an Epilepsy patient. We began by looking for apoptosis using the TUNEL assay with the cell lines mentioned above using dose response to evaluate the percentage of cell death compared to the controls. We were able to conclude that a dose of 4 millimolar to 8 millimolar was able to produce efficient apoptosis in more than 90% of the GBM cell lines. These doses affected around 20% of the white matter cells. To evaluate the effect of early apoptosis, we re-evaluated this phenomenon using the Caspase-3 assay in the two cell lines that we would subsequently use in our in vivo work; LN229 and U87 (figure 1). By using 4 millimolar and 8 millimolar 3-BrPA, we were able to show an increase of Caspase-3 in U87 and LN229 respectively. Using this information, we established an in vivo rat flank tumor model to translate our results from in vitro to in vivo. We did two pilot experiments, one with LN229 and the other with U87.

Discussion Continued

We implanted the cells using Matrigel into the right flank of each rat. The control group was treated with intra-tumoral injections of PBS every three days for twenty one days and the treatment group was injected with 3-BrPA in a PBS vehicle for the same time period.

Figure 1

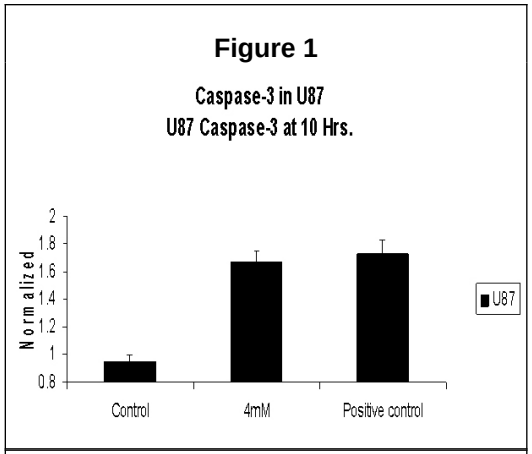
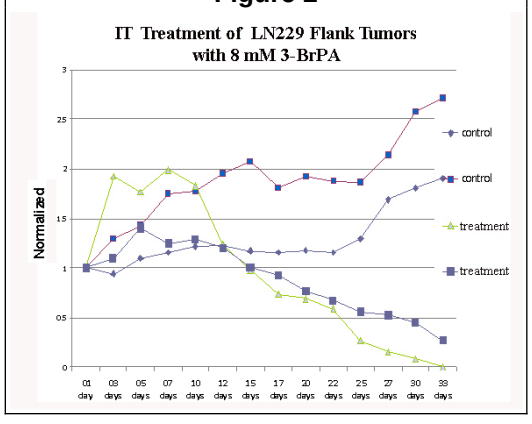


Figure 2



Results

Cells were treated with 3-BrPA at doses ranging from 500 micromolar to 15 millimolar for 48 hours. TUNEL analysis showed evidence of apoptosis in more than 95% of GBM cells (all cell lines) following 3-BrPA treatment at any dose, compared to HA which showed 20% apoptosis ($P<0.005$). In vivo experiments showed decreased tumor size in treated rats over the course of injections while tumor size increased in control group (figure 2). After stopping treatment, tumors continued to decrease in size in the treated animals and have not returned after three months. Treated animals showed tumor regression without apparent toxicity or recurrence. These findings attest to the feasibility of destroying advanced, highly glycolytic brain tumors. We feel that the possibility of controlling the progression of brain tumors through changes in the metabolic environment should be studied in depth. We will continue to explore the potential impact this could have on treating brain tumors.

Acknowledgements

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