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Characterizing and inhibiting two pathways activated in Glioblastoma Multiforme

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Despite major improvements in imaging, radiation, and surgery, the prognosis for patients with Glioblastoma multiforme (GBM) remains clinically challenging. New treatment strategies are badly needed to reduce the mortality and morbidity associated with this disease. The resistance of these tumors to conventional treatments makes GBM patients ideal candidates for molecularly targeted therapies and several agents are currently being developed(1). Because GBM is genetically heterogeneous, combination therapies or the use of multikinase inhibitors are more likely to achieve the greatest therapeutic benefit(2,3). However, genes that can be productively targeted for effective therapies in patients remain to be identified. The overall objective of this project was to better understand the signaling pathways driving cell survival so that new targets can be identified in gliomas. These studies will lead to an increased understanding of the proteins that are altered in this disease and should provide promising opportunities to develop better treatment strategies based on specific molecular targets.

Two parallel pathways, which are both activated in GBM, converge on downstream survival signaling cascades. Studies have demonstrated that blocking only one pathway often leads to a transient response (e.g., delayed time to progression), but tumors eventually progress(4). More effective therapies are likely to be those that inhibit more than one target or pathway(5). Targeting anti-apoptotic Bcl-2 proteins in combination with RAS/MAPK or AKT/mTOR inhibition is a rationale approach.

To determine if inhibiting both the RAS/MAPK and AKT/mTOR pathways in combination results in increased apoptosis in glioma cells, I compared the level of apoptosis in cells treated with each inhibitor alone and in combination. Treatment of glioma cells with a MEK inhibitor in combination with a PI(3)K inhibitor has not previously been reported and therefore represents a new approach in the field. We already know that just inhibiting RAS/MAPK or AKT/mTOR alone results in cell cycle arrest but not death. I tested the effect on cell death when combining the inhibitors of both pathways, and saw an increase in cell death. I determined the growth inhibitory and apoptotic sensitivity of several human glioma cell lines to inhibition of both RAS/MAPK and AKT/mTOR pathways. Due to the heterogeneous nature of GBM, I predicted and saw that these cell lines display varying levels of sensitivity to MEK/PI(3)K inhibition. These differences can then be used in the future to further define the mechanism(s) by which the AKT and MAPK pathways mediate survival signaling in glioma cells.
Characterizing and Inhibiting Two Pathways Activated in Glioblastoma Multiforme

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INTRODUCTION

Despite major improvements in imaging, radiation, and surgery, the prognosis for patients with Glioblastoma Multiforme (GBM) remains clinically challenging. New treatment strategies are badly needed to reduce the mortality and morbidity associated with this disease. The resistance of these tumors to conventional treatments makes GBM patients ideal candidates for molecularly targeted therapies and several agents are currently being developed(1). Because GBM is genetically heterogeneous, combination therapies or the use of multidrug inhibitors are more likely to achieve the greatest therapeutic benefit(2,3). However, genes that can be specifically targeted for effective therapies in patients remain to be identified. The overall objective of this project was to better understand the signaling pathways that are activated in GBM, and to identify small molecules that can block these pathways.

METHODS

A high-throughput system (SuperArray CASE ELISA) was used to determine the optimal concentrations of inhibitors to decrease phosphorylation. The system was slightly modified after a 48 hour (BEZ235) and 72 hour (CI-1040) treatment period. Western Blots were used to verify the inhibition of phosphorylated proteins in the pathway (P-ERK, P-AKT, P-p70s6K, and P-MEK) and reprobed for total protein and -tubulin. Flow cytometry was used to determine cell-cycle arrest and apoptosis.

RESULTS

The two inhibitors used were CI-1040 (Philips), which is a 2nd generation MEK inhibitor, and BEZ235 (Novartis), which is a Class I PI(3)K inhibitor. These inhibitors were used alone and in combination to determine the level of apoptosis and cell death in various human GBM cell lines, SF-268, SF-295, SF-538, SNB19, SNB75, and U251. The data obtained also show significant "cross-talk" between the two pathways, which could also be affecting the combination's ability to work as effectively as expected. Specifically, an increase in phosphorylation and apoptosis was observed in combination than the single agent studies indicated.

CONCLUSIONS

The main conclusion that can be drawn from these experiments is that the CI-1040 and BEZ235 compounds do inhibit the respective pathways, but there is something in the cell that is keeping the combination of them from inducing complete apoptosis. Different concentrations may need to be used in combination to complement the single agent studies indicated.

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