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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.
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 patient 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 multisite inhibitors are more likely to achieve the greatest therapeutic benefit (2). However, genes that can be exclusively targeted for effective therapies in patients remains to be identified The overall objective of this project was to better understand the signaling pathways driving cell survival 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 identify new treatment strategies based on specific molecular targets.

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Inhibitors used:
- CI-1040 (10 µM)
- BEZ235 (0.1 µM)
- ERK
- AKT

RESULTS

A flow cytometer (Guava) was used to determine cell apoptosis and G1 growth arrest to see if the inhibitors were killing the GBM tumor cells. Although there wasn't a large amount of apoptosis (sub G1 - brown), there was a visible increase in cell death and cells present in the G1 phase (green) when compared to the control cells and cells treated with the combination of drugs. The green value represents G0/G1 phase in the cell cycle.

FUTURE DIRECTIONS
Different concentrations of inhibitors can be tested to see if higher concentrations are necessary when combined. There are also newer generations of compounds being released that may hit their targets more effectively and be more stable in the cell than the ones tested here. Other proteins known to be involved in apoptosis will be examined, like the anti-apoptotic Bcl-2 family members, including Bcl-2 and Bcl-xl. If these can be inhibited, cell death may be achieved more fully. siRNA will also be performed to completely knock out phosphorylated (activated) proteins. Some possibilities to target all include three isoforms of Raf (A, B, and C) and PI3K, which disabling the pathways for cell survival.

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REFERENCES