The synergistic effect of radiation and inhibition of SPAK/OSR1 in the reduction of cell proliferation and clonogenic potential in patient-derived GBM cells in vitro.

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Abstract

Glioblastoma (GBM) is the most aggressive brain tumor in adults. Cell invasion, migration and proliferation into the heathy brain parenchyma it's one of the most important challenges in the treatment of this deadly tumor. One potential mechanism that GBM cells can utilize to enhance cell migration and evade pro-apoptotic signals is the tight regulation of cell volume by the STE20/SPS1-Related Proline-Alanine-Rich Protein Kinase (SPAK) and (Oxidative Stress Responsive Kinase 1) OSR1 kinases. Dynamic changes in cell volume can be used by GBM cells to disseminate through the narrow perivascular spaces of the brain. In addition, cancer cells could counteract pro-apoptotic reduction of cell volume by increasing the activity of these kinases. The objective of this project is to test the efficacy of SPAK and OSR1 inhibition alone or in combination with radiotherapy. For this purpose we evaluated the impact of this novel therapy on the proliferation, clonogenicity and apoptosis of primary patient-derived GBM cells in vitro. To achieve our goal we tested a novel SPAK/OSR1 inhibitor (a small molecule called YU566) in two patient derived GBM lines. Cell proliferation and colony formation were determined after treatment using 1uM YU566 alone or in combination with radiotherapy (at different doses 2, 4 Gray (Gy)). We found that radiation and inhibition of SPAK/OSR1 could act in a synergistic fashion, decreasing cell proliferation and clonogenic potential. The next steps in our research will be to determine the mechanisms of cell death and the implications of this therapy in vivo.

Background

- Abundant cell invasion and migration through the brain parenchyma is a hallmark of GBM.
- In the brain, GBM cell invasion can be regulated by cell volume changes. Cell volume dynamics can facilitate invasion and regulate cell division.
- SPAK and OSR1 are members of the STE20 superfamily of MAPK kinases. These kinases are master regulators of cell volume regulation.
- We decided to study their implication in GBM malignancy by targeting their activity using small molecule inhibitors. Specifically we want to investigate if radiation treatment in combination with SPAK/OSR1 inhibition has synergistic effects.

The clonogenecity of 965 GBM cells after the treatment of 1uM YU566 followed by radiation.

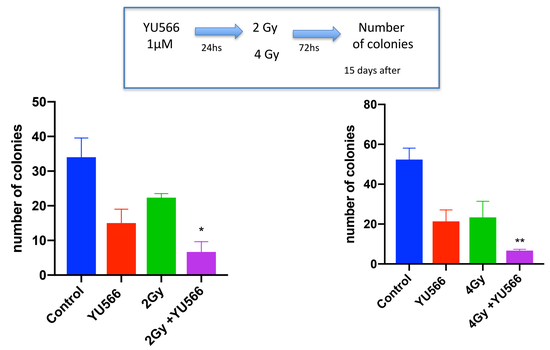

Figure 1. Clonogenecity of 965 GBM cells after the treatment of 1uM YU566 for 24 hours followed by radiation after 72 hours. The different treatment groups are shown as control, YU566, Radiation and the combination of both. The graph on the left reveals radiation at 2 Gy. The graph on the right reveals 4Gy radiation treatment. Combination treatment of 2Gy + YU566 p=0.0137; Combination treatment of 4Gy + YU566 p=0.0065.

Cell Proliferation of 120,612 and 965 GBM cell lines using Yu566 indifferent concentrations in combination with radiation.

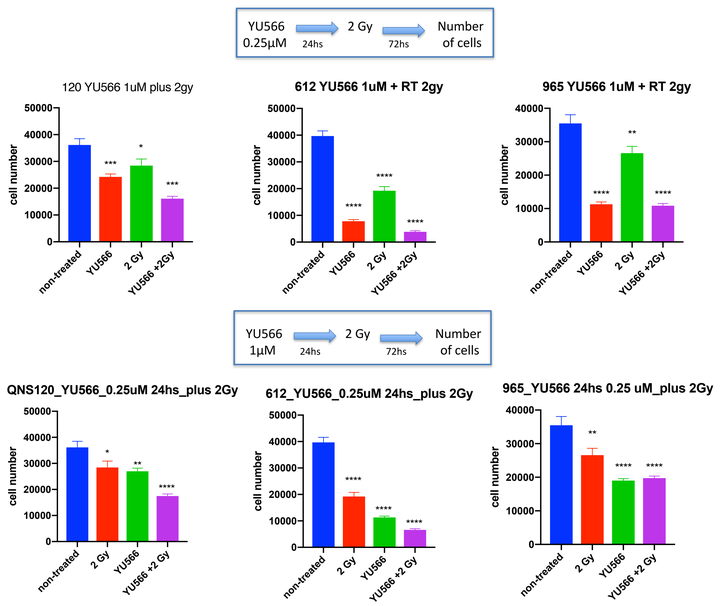

Figure 2. Cell proliferation of GBM cells using YU566 inhibitor in combination with radiation treatment using three cell GBM lines: 120, 612 and 965. The upper panel consisted of .25 uM YU566 while the lower panel consisted of 1 uM YU566. Bar graph represent mean plus SEM, all groups are compared to non-treated control. ****p<0.0001 ***p=0.0002 **p=0.0021 *p=0.0332.

The clonogenecity of 965 GBM Cells after the Treatment of 2Gy versus 4Gy Radiation Followed by 1uM of YU566

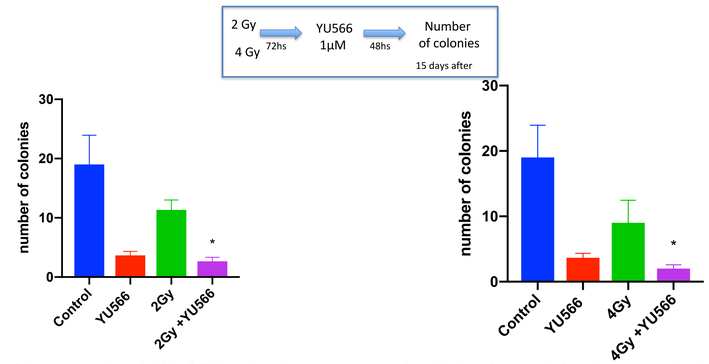

Figure 3. Clonogenecity of 965 GBM cells after treatment of radiation first at 2 Gy shown on the left versus 4 Gy on the right followed by inhibition via 1 uM of YU566. The different treatment groups are shown as control, YU566, Radiation and the combination of both. The graph on the left reveals radiation at 2 Gy, the graph on the right reveals 4Gy radiation treatment. p= 0.0332, p=0.0142, respectively.

Methodology and Statistics

Cell proliferation was measured using Cyquant assay after combined treatment of radiotherapy and inhibition of SPAK/OSR1. YU566 was added to the cells for 24 hours followed by radiation for 72 hours in three GBM cell lines: 612, 120, and 965. (Figure 2). A parametric one way-ANOVA, was then run to determine significance. Dunnett's test was then run in order to do a multiple comparison between the different treatments. All treatment groups were compared to the control.

Colony formation was measured to determine clonogenecity after 15 days of treatment. 1 uM of inhibitor YU566 was added for 24 hours followed by radiation for 72 hours. This tested the capability of cell growth clones. The effects were then observed and measured after 15 days after treatment where the number of colonies were counted (Figure 1).

Colony formation was again tested however by radiation first for 72 hours followed by 1 uM of the inhibitor YU566 for 48 hours. This was done to determine if the order of radiotherapy versus inhibition of SPAK/ OSR1 first resulted in a shift in results. Cell clonogenicity was measured again after 15 days of treatment (Figure 3).

Conclusions

Glioblastoma (GBM) is the most aggressive brain tumor in adults. The five-year survival rate for GBM is less than three percent and is mostly attributed to GBM's ability to uncontrollably proliferate along with its aforementioned ability to infiltrate into brain tissue necessary for survival that is not able to be surgically removed. After the use of statistical tests, a significant decrease in proliferation was found when the treatment consisted of inhibition and radiation in combination. The different cell lines revealed different percent differences; however, the pattern was the same in all cell lines for the reduction of cell proliferation. The chologenicity revealed similar patterns with the inhibitor having a more significant impact alone than radiation alone.