Investigating Effects on Cell Cycle Progression in Treatment-Resistant Medulloblastoma Cells

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Abstract

- Medulloblastoma is the most common childhood brain tumor.
- These tumors occur in the cerebellum in the posterior fossa.
- Alterations in the Sonic Hedgehog pathway (SHH) are present in ~30% of medulloblastomas and cause poor outcomes.
- Aggressive and treatment-resistant SHH-driven medulloblastomas are found to exhibit increased activation of pathways that cause increased glycolysis.
- Cancer cells utilize glycolysis to provide building blocks and energy for cell growth and proliferation.
- Fructose-2,6-bisphosphate is a key regulator of glycolysis by activating the enzyme PFK-1. F26BP is produced by the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase enzymes (PFKFB1-4).
- Previous studies from our lab have found that the PFKFB4 enzyme is highly expressed in medulloblastoma.
- Our lab has previously produced SHH-driven medulloblastoma cell lines that are resistant to an SHH pathway inhibitor (SHH-R cells) . These cells show higher PFKFB4 and proliferate faster than the parent SHH-driven cells that are sensitive to the SHH inhibitor (SHH-S cells).

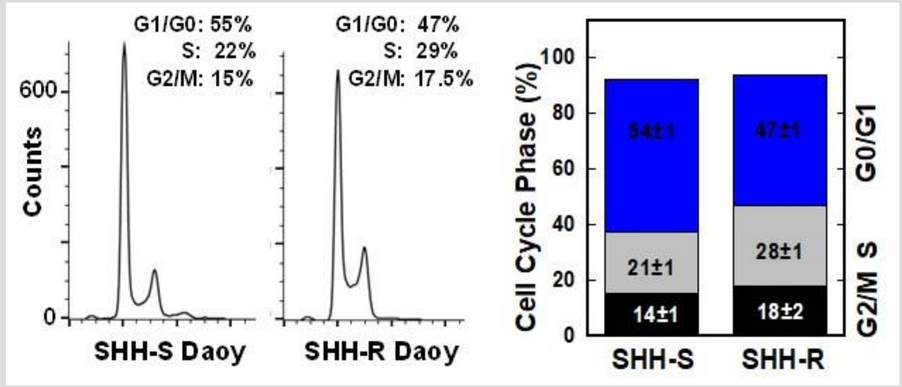
Objective

The objective of this study is to compare cell cycle progression in SHH –S and SHH-R medulloblastoma cells and to examine effects of PFKFB4 inhibition on cell cycle progression in these cell lines.

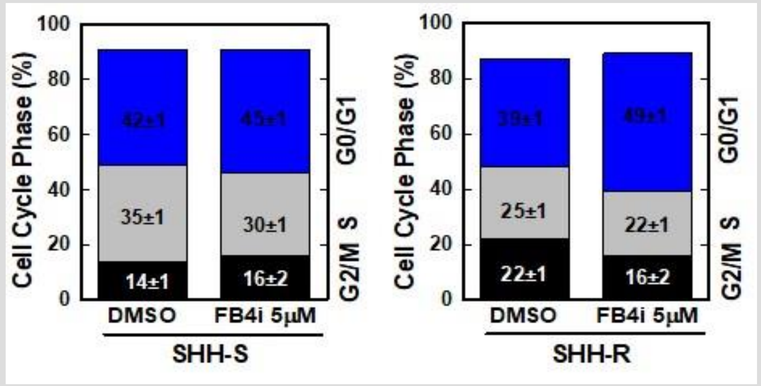
Hypothesis

We hypothesize that SHH-R medulloblastoma cells exhibit altered cell cycle progression relative to SHH-S cells and that these SHH-R cells will be more sensitive to the effects of PFKFB4 inhibition on the cell cycle.

Results



SHH inhibitor resistant medulloblastoma cells show more rapid cell cycle progression than SHH inhibitor sensitive cells.



SHH-R cells are more sensitive to the effects of a PFKFB4 inhibitor (FB4i) on cell cycle progression than SHH-S cells.

Methods

- Equal numbers of medulloblastoma cells were plated in 6 well plates and exposed to DMSO (as vehicle) +/- increasing concentrations of a PFKFB4 inhibitor (FB4i).
- To harvest cells, wells were washed with phosphate buffered saline (PBS), detached with trypsin and pelleted and pellets washed with PBS.
- Cell pellets were resuspended in 100 µL PBS and ice-cold 70% ethanol was added to fix the cells. The cells were then stored at -20°C overnight.
- Samples were centrifuged, ethanol aspirated and the cells were vortexed.
- Propidium Iodide (in PBS) was added to the cells and samples were passed through 25 gauge needles 2-3 times to break up clumps and incubated for 30 min at 37°C in the dark.
- Finally, the cells were inserted into the flow cytometer in order to read the number of cells found in each phase (G0/G1, S , G2/M).

Future Directions

We plan to examine effects of PFKFB4 inhibition on cell cycle progression further in these cell lines and in cells from patients with treatment-sensitive and treatment-resistant medulloblastomas

Acknowledgements

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