Modulation of U87 Glioma Stem Cells Using a Small Molecule Wnt Inhibitor

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Introduction

Glioblastoma multiforme (GBM) is a rare but aggressive primary brain tumor. Its dismal prognosis and high rates of recurrence have been in part attributed to the presence of glioma stem cells (GSCs). While many studies have been done on the signaling pathways, including the Wnt pathway, of these GSCs, there has not been a viable small molecular inhibitor that targets this subpopulation of cells. Our study aims to explore the use of a novel small molecule Wnt inhibitor, C59, to target GSCs.

Methods

We subjected U87 cells to increasing concentrations (0-200nM) of C59 for 48 and 72h, and quantified the effect of the drug by measuring the number of tumorspheres per field (at 40X) and the average diameters of these tumorspheres. Changes in proliferation were subsequently quantified using the MTT assay. Finally, treated cells were analyzed using flow cytometry for changes in proliferation and apoptotic levels with BrdU and Annexin V as markers respectively.

Results

At 72h, the number of tumorspheres formed per field and the average diameter of the tumorspheres both showed a dose-dependent decrease (Fig.1). The MTT assay also showed a significant decrease in proliferation at 72h for C59 concentrations greater than or equal to 100nM (Fig.2), which was supported by flow cytometry analyses of BrdU incorporation (Fig.3). Interestingly we saw a concurrent increase in early apoptosis measured by flow cytometry.

Effect of C59 on Tumorsphere Growth

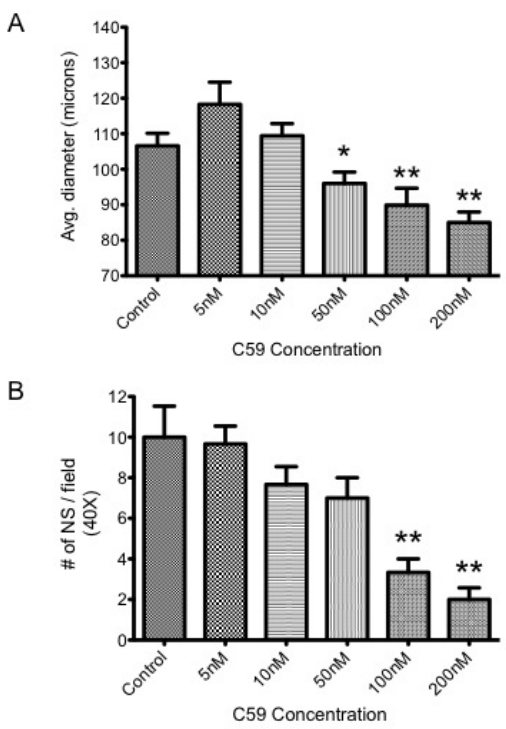

Fig.1 (A) Average diameter of tumorspheres decrease in a dose-dependent manner. One-way ANOVA: P<0.001. Newman-Keuls Post Test: *P<0.05, **P<0.01 (B) Number of tumorspheres per 40X field also decreased in a dose-dependent manner. One-way ANOVA: P<0.001. Newman-Keuls Post Test: **P<0.01

Conclusions

Our results showed that treatment of U87 GSCs with 100nM of C59 for 72h is sufficient to decrease tumorsphere formation, both in number and size. This decrease could be attributed to a combined effect of decreased proliferation and increased apoptosis of the GSCs through inhibition of the Wnt pathway. Together, these findings suggest a potential for C59 to be used as a small molecule inhibitor for GSCs.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of a small molecule Wnt inhibitor in GSCs, 2) Discuss, in small groups, approaches to targeting GSCs, 3) Identify an effective adjuvant treatment for GBM.

MTT Assay After C59 Treatment

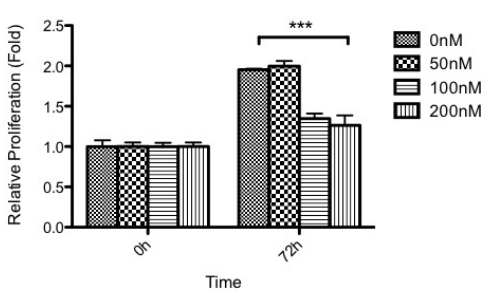

Fig.2 MTT Assay used to measure proliferation after C59 treatment showed a significant decrease at concentrations above 100nM. Two-way ANOVA: P<0.001

Flow Cytometry Analyses of C59 Treatment

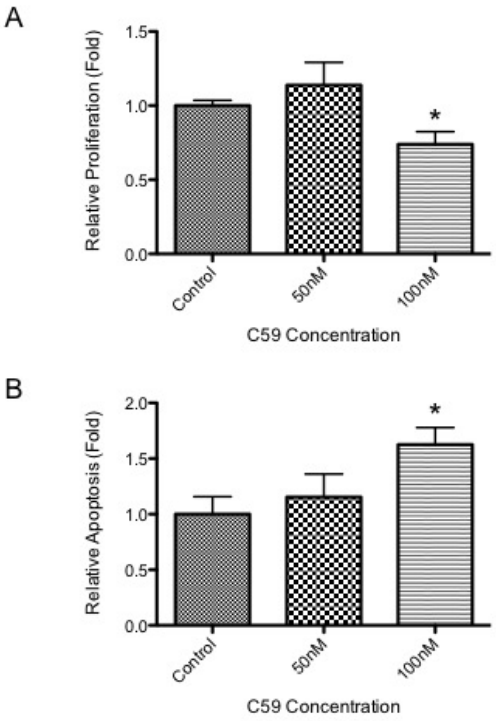

Fig.3 (A) Flow cytometry analysis of BrdU incorporation was used to quantify percentage of cells which were undergoing proliferation. A significant decrease was observed after 72h of C59 treatment at 100nM. Student T-test: P=0.030 (B) Annexin V was used as a marker for early apoptosis. A significant increase was observed in U87 cells treated with 100nM of C59. Student T-test: P=0.047