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Natural Products as Therapeutic Agents in Cancer Treatment

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STATEMENT OF THE PROBLEM

Background

Cancer accounts for 25% of deaths in the United States.[1] Brain tumors play a large role in this percentage. The brain tumor incidence rate was 6.5 per hundred thousand between the years 2006 and 2010 in the United States.[2] Statistics indicate that there is an age adjusted mortality rate of 4.3 per hundred thousand persons across all age groups diagnosed with brain and other nervous system invasive cancers.[2]

Flavonoids and curcuminoids have shown potential for treating brain tumors in past studies. Genistein, a flavonoid, has shown promise for treating brain tumors since it inhibits the growth of glioblastoma and medulloblastoma cells by stopping the cell cycle at the G2/M checkpoint.[3] Another study found that the curcuminoid curcumin binds to the cell surface membrane and later goes into the cytoplasm to induce cell death.[4] A separate study found that curcumin downregulates various IGF (insulin-like growth factor) ligands to inhibit glioblastoma and medulloblastoma cells.[5] Despite these promising results, there are some major gaps in the research. Specifically, there has not been a significant amount of research done on the effectiveness of flavonoids in the treatment of brain tumors.

Significance of the Problem

Relative to other types of cancers, brain tumors are difficult to treat. They prove resistant to chemotherapy and radiotherapy and show an increased tendency for infiltration. In response, scientists have conducted research to reveal the nature of brain tumor cells and the relationship they have with their *in vivo* environment. In one study, researchers found that brain tumor stem cells live in a unique environment. They depend on perivascular cells within the brain which provide an ideal growth environment for these stem cells.[6] These researchers also discovered that brain tumor cells depend on microRNAs for growth, proliferation, and overall survival.[6] Some avenues of current brain tumor research attempt to target these microRNAs in hopes of discovering a more effective clinical treatment.[6]

Past research has studied the effects of certain plant compounds such as curcuminoids and cyanidins on various types of cancer cells. These compounds were found to inhibit metastasis in breast and lung cancer cells.[7,8] Our work will attempt to build and expand upon prior research examining flavonoids' effect on brain tumors in an attempt to find an effective treatment.

OBJECTIVES

Objective 1: To determine the effectiveness of flavonoids as therapeutic agents in brain tumor treatment.

Objective 2: To identify the signaling mechanism by which flavonoids mediate their therapeutic effects on brain tumor cell lines.

HYPOTHESES

Alternative Hypothesis 1 for Objective 1: There is a difference in cell death between cells treated with flavonoids and control cells not given any treatment.

Alternative Hypothesis 2 for Objective 1: There is a difference in cell proliferation between cells treated with flavonoids and control cells not given any treatment.

Alternative Hypothesis 3 for Objective 1: There is a difference in brain tumor invasion between cells treated with flavonoids and control cells not given any treatment.

PROPOSED METHODS

Study Design
Controlled experimental study

Sample
Human brain tumor cell lines: U-1242, U-251, and U-87

Treatment
Flavonoids at 10, 20, 40, and 80 µM

Data Collection
Experiments will be performed in triplicate unless further trials are needed to resolve discrepancies found in initial three experiments. Data from flow analyses and Western Blot analyses will be saved as JPEG files and printed. Data from MTT assay and cell count will be recorded in laboratory notebooks. Notebooks will be checked and witnessed by fellow research participants. Data will be inputted into IBM SPSS 21® as appropriate.

Measurements

Cell count
Cells will be stained with trypan blue and counted using the grid count method and a hemocytometer.[9]

Cell proliferation
MTT Assays will be utilized to determine how flavonoids influence the proliferation of brain tumor cells.[10] Western blot analyses will be conducted to identify proliferation markers such as MAPK, Akt, EGFR, and PKCs.[11]

Cell death
Samples will be prepared and analyzed using flow cytometry to measure cell growth.[10] Western blot analyses will be conducted to identify apoptotic markers such as cleaved Poly ADP ribose polymerase (PARP) and cleaved caspases.[11]

PROPOSED ANALYSES

IBM SPSS 21® will be used to conduct all statistical analyses. Unpaired *t*-tests will be run to compare results from treated cells to control cells at 95% confidence. If necessary, one-way ANOVA with multiple comparisons will be used to compare multiple treatment groups and a control at 95% confidence. The Tukey test will be used to run post-hoc analyses if needed.

PROJECT TIMELINE

January 2014-December 2015: Data collection and analysis

LIMITATIONS

- While the study examines multiple brain tumor cell lines, the generalizability of the study is limited as there are many more cell lines found in brain tumors.
- Results obtained in the *in vitro* environment may not translate to the *in vivo* environment.

FUTURE DIRECTIONS

The goal of this study is to provide the basis for future *in vivo* translational studies in animal models.

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