The Effects of cAMP on the Invasion of Glioblastoma Neurospheres

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Introduction

Glioblastoma Multiforme (GBM) is a type of brain tumor that displays highly aggressive invasion, making surgical resection nearly impossible.

A promising category of GBM therapeutic agents are activators of cyclic mediated adenosine monophosphate (cAMP)[1].

The outcome of cAMP signaling is dependent on where it is located subcellularly, or it's microdomain[2,3]. cAMP production at subcellular sites is controlled by adenylyl cyclases in different parts of the cell[4].

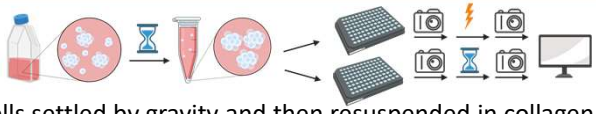
Here, we study cAMP production isolated to the cytoplasm using the photoactivatable adenylyl cyclase, bPAC[3]. To observe the effects of universal cAMP production activation, the pharmacological agent forskolin (FSK) was used[5,6].

Invasion was observed in patient-derived GBM neurospheres instead of traditional 2D culture. Neurospheres more accurately represent tumor behavior in vivo because of their heterogeneous cell population[7].

Project Goal

This study will explore 1) how the change in cAMP expression at the cellular level effects behavior of primary cell-based tumor and 2) if bPAC is successful at inducing those behavior changes.

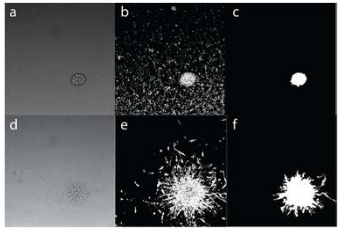
Neurosphere Assay



Cells settled by gravity and then resuspended in collagen. Imaged at 0hr and 24hr. For light exposed conditions, 488nm light was pulsed for 1s every minute for 24 hours.

Results

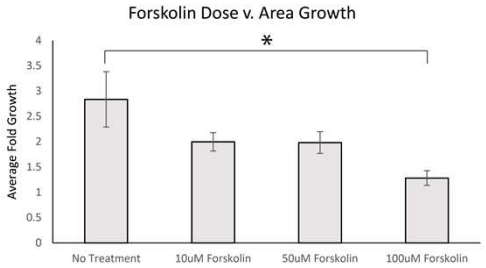
Neurosphere Image Analysis



Analysis was performed in MatLab using edge detection.

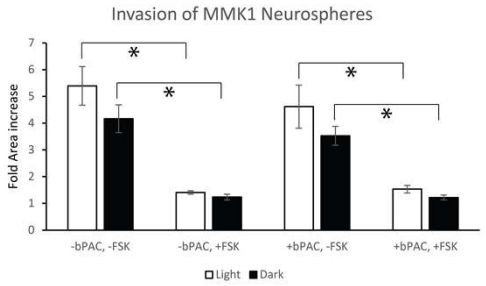
$$\frac{(24\ hr\ area)(0\ hr\ area)}{\#\ neurospheres\ in\ field}$$

Optimization of Forskolin Dosage



MMK1 neurospheres were seeded in collagen containing forskolin and allowed to invade for 24 hours.* indicates a student t test < 0.05.

Effects of bPAC and FSK on Neurosphere Invasion



MMK1 neurospheres in the light and dark conditions were allowed to invade a collagen matrix for 24 hours. Forskolin was used at 100µM. bPAC cells were created through viral transduction. * represents a student t-test < 0.05.

Discussion

Forskolin treatment significantly decreased neurosphere invasion. This data supports the hypothesis that cAMP is involved in glioblastoma survival.

bPAC expressed in the cytosol had no significant effect on neurosphere invasion.

It is possible that the methods of light exposure in this study were not strong enough to trigger high bPAC activation. This is supported by the lack of significant change in invasion between the light and dark conditions. Further studies should explore how time of illumination or light intensity affects bPAC activation and subsequent cAMP production.

It is also possible that invasion behavior is only significantly impacted by cAMP generation localized to areas not covered by this study. This study should be repeated using bPAC that is site specific to the outer mitochondrial membrane, nucleus, or plasma membrane.

Conclusions

1. Treatment with 100µM forskolin significantly decreased invasion and growth in MMK1 neurospheres
2. bPAC localized in the cytosol has no significant effect on MMK1 neurosphere invasion with or without exposure to light

References and Funding

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This work was supported by a Summer Undergraduate Research Fellowship and NIH award R01- NS103486 (Lawrence)