Dexamethasone-Mediated Activation of Fibronectin Matrix Assembly Inhibits Dispersal of Human Primary GBM Cells in a Novel in Vivo Model

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Introduction

Early and continuing dispersal of tumor cells away from the surgical margin is a major cause of recurrence and relapse of GBM patients. Identifying drugs that can mitigate dispersal, particularly after patients undergo the Stupp protocol, may increase the length of time to radiological recurrence and improve overall survival. Previous studies from our lab have shown that Dexamethasone (Dex), a drug currently used to treat brain tumor related edema, but which is tapered as soon as possible after surgery, can also significantly reduce dispersal of primary human GBM (pGBM) cells in vitro and ex vivo. Here, we introduce a novel in vivo dispersal assay to demonstrate that Dex also significantly inhibits dispersal in vivo.

Methods

Single cell dispersions of fluorescently-labeled pGBM cells were injected into mouse retinas and mice were either treated with vehicle control or Dex at a dose equivalent to 8mg/day for 5 days, whereupon mice were sacrificed and eyes removed and fixed in 4% PFA for 24 hours. Retinas were then extirpated, stained with Fn antibody and DAPI, mounted, and imaged by scanning laser confocal microscopy. z-stacks were collected and penetration

Figure 1

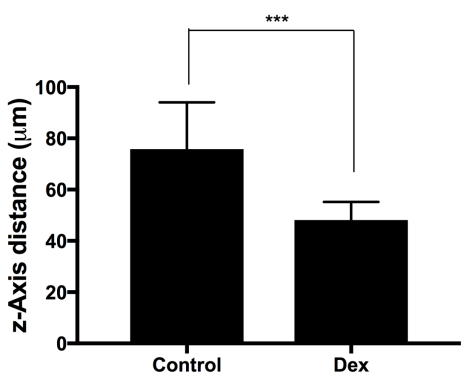

Figure 1. Z-axis penetration of Dex treated() pGBM vs controls(). *** represents significant difference by Student t-test, p= 0.0005.

Figure 2

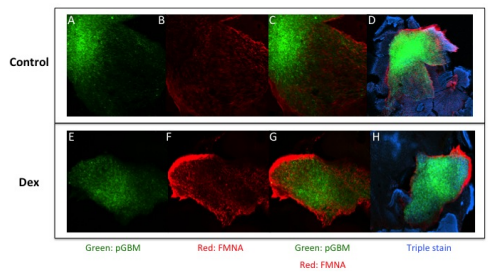

Figure 2. Fibronectin Matrix Assembly of Mouse Retinas for Control and Dex-Injected Mice. (A-D) Vehicle control. A) pGBM cells stained with PKH-2 fluorescent membrane dye. B) Unorganized fibronectin stained with Fn antibody. C) Cords of pGBM cells infiltrating along unorganized fibronectin. D) Low power field of triple stained retina (green: pGBM, red: FNMA, blue: dapi counter stain). pGBM cells are seen infiltrating into the surrounding retinal tissue. (E-H) Dex treated mouse. E) pGBM cells stained with PKH-2 fluorescent membrane dye. F) Fibronectin organized into a matrix demonstrated by Fn antibody. G) Activated fibronectin matrix assembly (FNMA) by pGBM cells results in containment of the tumor mass. H) Low power field of triple stained retina (green: pGBM, red: FNMA, blue: dapi counter stain). GBM cells are contained within a barrier of FNMA.

References

Results

We show that Dex significantly reduces z-axis penetration of GBM cells into mouse retina (Figure 1), that Dex treatment significantly alters the morphology of dispersal of injected GBM cells within the x,y plane (Figure 2), that without Dex, the presence of fibronectin effectively increases tumor cell dispersal, that Dex treatment activates fibronectin matrix assembly (FNMA) by GBM cells leading to containment of the tumor mass, and that Dex-mediated activation of FNMA is fibronectin dose-dependent.

Conclusions

Our study defines a role for fibronectin as a facilitator of GBM dispersal and Dex-mediated activation of FNMA as an inhibitor of that process.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the potential of dexamethasone to inhibit tumor cells dispersal in an in vivo model. 2) Discuss in small groups the role of dexamethasone and fibronectin matrix assembly in tumor cell dispersal. 3) Identify dexamethasone as a potential treatment in the management of GBM cell dispersal.