Nanocarrier Therapy for Treating Invasive Gliomas

Jennifer Munson[1], Eleanor DeHitta[1], Rania Khan[1], Levi Fried[2], Arthur Iskhakov[2], Jack Arbiser[2], Ravi Bellamkonda[1]
[1]Georgia Institute of Technology, [2]Emory University Medical School

Statement of Purpose: Invasiveness of brain tumors is a major reason for poor prognosis after diagnosis. Unfortunately, there candidate drugs that negatively impact invasive gliomas are not available. Here, we screened 24 natural compounds using *in vitro* assays with a rat glioma cell line to identify a drug that is truly anti-invasive without necessarily being cytotoxic. Additionally, gliomas present a difficult drug delivery paradigm due to the blood brain barrier. Our laboratory has recently demonstrated that 100nm-scale liposomes take advantage of the enhanced permeability and retention of tumors by selectively accumulating there [1]. We have fabricated a nanocarrier system to deliver the anti-invasive agent *in vivo* using an aggressive tumor model.

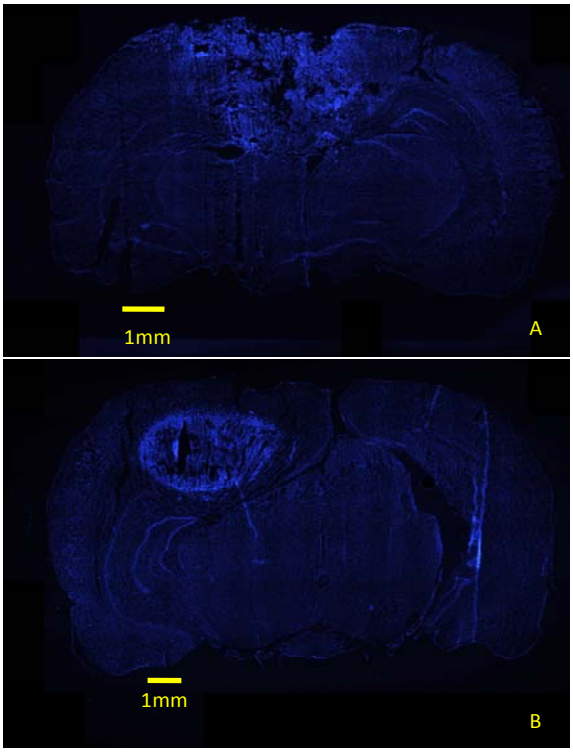

Figure 1: Tumor progression at Day 11 in Fischer 344 rats using DAPI stain for cell nuclei. A) untreated tumor shows chaotic borders and infiltrative tumor cells. B) IB-liposome treated tumor shows a more encapsulated morphology with well-defined border between tumor and healthy brain.

Methods: *In vitro* compound screen: An aggressive rat astrocytoma cell line, RT2, was maintained *in vitro*. This cell line was used to test 24 compounds at varying concentrations in cytotoxicity and proliferation assays using CCK8. Compounds without detrimental effects on viability and growth were tested using the Boyden chamber invasion assay. Compounds were also tested for viability against astrocyte cultures. Of the 24 compounds screened, Imipramine Blue (IB) was most effective as an anti-invasive agent.

Nanocarrier synthesis: Liposomes were made from DSPC (85mol%), DSPE-PEG (5mol%), and cholesterol (10mol%) by dissolving the lipids and 2mg/ml of IB in ethanol. The solution was hydrated using phosphate buffered saline at 70°C to yield liposomes. Liposomes were extruded to a size of 160nm as assessed by dynamic light scattering. Unbound drug was removed via sepharose column separation and then diafiltrated to a final IB concentration of 3.5 mg/ml.

***In vivo* animal studies:** For tumor inoculation, 8-10 week old Fischer 344 rats were anesthetized using 2-3% Isoflurane inhalant. A 1 cm incision to the skull was made and a 2mm burr hole was drilled 2mm lateral and 6mm posterior to bregma. 250,000 cells were injected in 10µl of Leibovitz media over a period of 10 minutes using a Hamilton syringe. The hole was filled with bone wax and the wound closed. On days 4 and 7 of tumor growth, 16 mg/kg (IB concentration) of IB-liposomes were injected via the tail vein. On Day 11 following tumor inoculation animals were sacrificed by intracardial perfusion. Brains were removed, photographed, fixed, embedded in OCT and sectioned. Slides were collected, stained using DAPI and imaged. Image analysis was conducted in a blinded fashion to quantify invasiveness.

Results: We have identified a novel compound, Imipramine Blue, as a potent anti-invasive agent for gliomas. We have then efficiently encapsulated IBin liposomal nanocarriers. In preliminary studies, IB-liposomes significantly impacted glioma invasion in vivo.

Conclusions: In this study, we have shown effective use of a novel anti-invasive compound, Imipramine Blue, to inhibit invasion *in vitro* and *in vivo* when delivered in nanocarriers. Therefore, anti-invasive compounds may be used for treatment of invasive tumors in conjunction with cytotoxic therapy or surgery. Further work will include co-encapsulation and delivery of this compound with common chemotherapeutics and use of this drug in conjunction with tumor resection. Additionally, work is underway to determine IB's mechanism of action.

References: 1. Karathanasis, E., *et al.* (2008) *Biomaterials* **29(**36**):**4815-22.