Minimally Invasive *In Vivo* Real-Time Identification of Human Astrocytoma with Sulforhodamine 101

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Abstract

Background: Specific brain tumor diagnoses depend on histopathologic interrogation of biopsies. Though helpful, current histopathological techniques lack the speed and specificity to be effective intraoperative diagnostic tools. Here we show that *in vivo* imaging of rodents implanted with human astrocytoma rapidly labeled with the physiological fluorophore sulforhodamine 101 (SR101) can immediately identify an astrocytic tumor from normal brain tissue.

Methods: Nude rats (n= 4) were stereotactically implanted with cultured human astrocytoma cells unilaterally. Five-weeks post implantation, rats were deeply anesthetized and underwent minimal bilateral craniotomies. SR101 (n=4) was applied subcortically through each craniotomy. An 0.85mm diameter fiber optic imaging bundle was attached to a FluoView 1000 confocal microscope, then passed through each craniotomy. Images were collected from the surface to a depth of five millimeters. Rodent brains were then extracted for *ex vivo* imaging. Fixed tissue was imaged following topical application of acridine orange (AO).

Results: Areas with tumor burden were immediately identified on the hemisphere implanted with tumor cells with SR101 labeling. Minimal SR101 labeling was appreciated within hemispheres contralateral to tumor implantation. Counterstaining with AO verified SR101 localized to areas of hypercellular astrocytoma.

Conclusion: SR101 rapidly and selectively labeled regions of human astrocytoma in rodent orthotopic xenografts. Coupled with *in vivo* imaging, SR101 shows utility for producing biopsy-free specific histological diagnoses. Clinical application of SR101 may provide immediate and specific intraoperative identification of astrocytic brain tumors.

Introduction

In neuro-oncology, specific tumor diagnoses are critical for development of treatment plans. Though multiple noninvasive imaging modalities exist, such as MRI, PET and CT scan, direct tumor sampling is often required for a definitive diagnosis. Sampling is typically achieved with either open biopsy or stereotactic needle biopsy. In open biopsy, a craniotomy is performed to directly visualize and biopsy a tumor sample. With fine needle biopsy (FNB), tumor is sampled through a small bur hole. Though less invasive, FNB suffers from greater missampling rates leading to acquisition of nondiagnositic tissue. Intraoperatively, frozen sections help determine if sampled tissue contains tumor, necrosis, or normal brain. However, definitive tumor diagnoses require specific stains which take several days to acquire. During this time, treatment plans for patients are delayed, as different pathologies require different treatment plans. Such is the case with astrocytoma compared to CNS lymphoma. These pathologies can appear similar with both noninvasive imaging and on frozen section, yet astrocytoma is best treated with surgical resection, and lymphoma is best treated nonsurgically with chemoradiation.

We propose developing a minimally invasive technique for generating rapid and specific *in vivo* intraoperative histopathologic diagnoses of astrocytic brain tumors by utilizing a physiologic fluorophore that discriminates astrocytoma from lymphoma, sulforhodamine 101. The development of fiber optic fluorescent imaging devices coupled with rapid and specific fluorescent biomarkers have had widespread utility in basic neuroscience. In this project, we model and investigate clinical application of these techniques on rodent orthotopic xenographs of human astrocytoma. We hypothesize *in vivo* imaging with a miniaturized fluorescent microendoscope will provide real-time tumor sampling and feedback which will allow immediate intraoperative identification of these malignant brain tumors.

Results

Minimally Invasive Fiber Optic Microendoscope

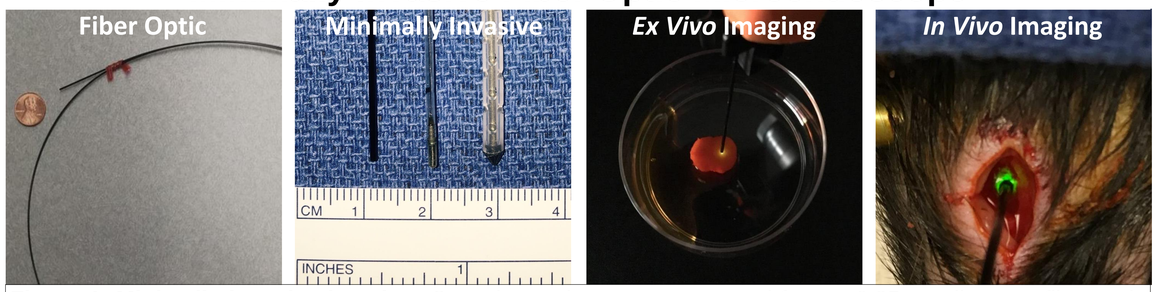

Figure 1. Minimally invasive imaging. Our microendoscope constructed from a 0.85mm diameter fiber optic bundle has a smaller cross-sectional area than a Camino ICP monitor (1.30mm) and ventricular catheter (3.00mm) which are routinely placed by neurosurgeons at bedside. The microendoscope permitted immediate *ex vivo* imaging of tissue biopsies and *in vivo* imaging through a miniature craniotomy.

Ex Vivo Imaging

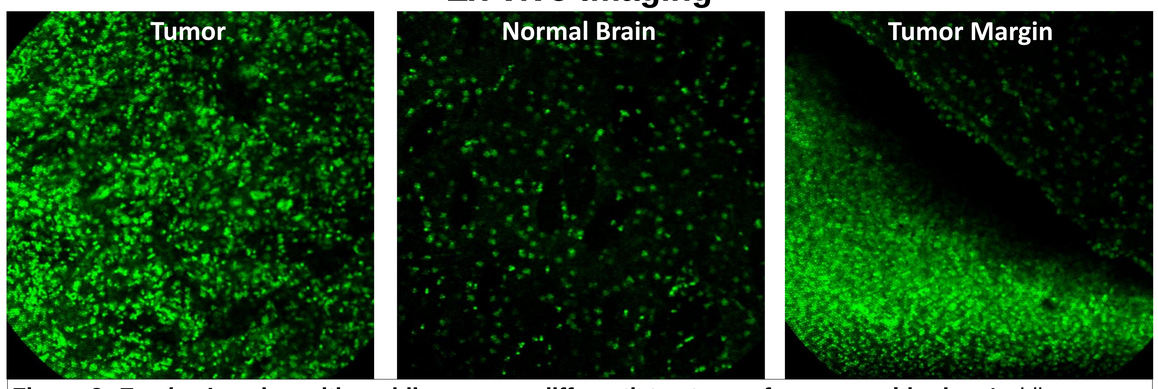

Figure 2. *Ex vivo* Imaging with acridine orange differentiates tumor from normal brain. Acridine orange immediately and nonspecifically stained cells within the field of view. Tumor regions were hypercellular compared to normal brain. Hypercellular and hypocellar tissue regions were visualized at the tumor margin.

Real-Time *In Vivo* Imaging

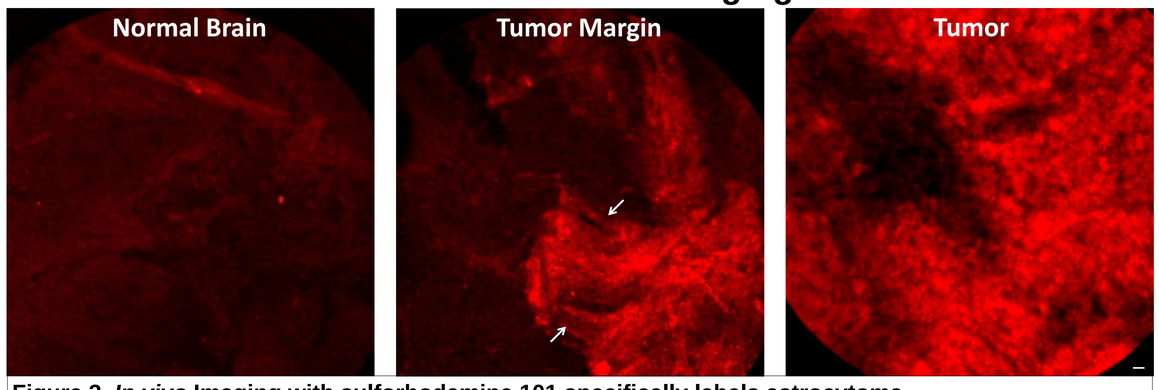

Figure 3. *In vivo* Imaging with sulforhodamine 101 specifically labels astrocytoma. Normal brain lacked areas with strong SR101 labeling. Regions with strong SR101 positivity were observed through the craniotomy ipsilateral to tumor implantation. SR101 labeling permitted real-time *in vivo* visualization of tumor margins (arrows) and regions of frank tumor. Scale bar equals 20*μm*.

Methods

Intracranial implantation: Nude rats (n=4) were anesthetized and placed in a small animal stereotactic headframe. A small incision was made to expose bregma. A burr hole was made 3.5 mm lateral to bregma. Human astrocytoma cells were infused at a depth of 4.5 mm below the surface of the brain, the burr hole was covered with bone wax and skin incision was sutured.

Craniotomy: Five-weeks following tumor implantation, animals were anesthetized and placed in a small animal stereotactic headframe. A 3mm diameter burr hole was made over the tumor implantation site and over contralateral normal brain.

Tumor labeling: To specifically label astrocytoma cells, twelve microliters of 50*μM* SR101 was injected at a depth of 3mm from the brain surface 20 minutes prior to imaging. Acridine orange (5mM) was applied topically to *ex vivo* tissue to nonspecifically label cells in the field of view for histological tumor verification.

Imaging: We constructed our fiber optic microendoscope by coupling a 0.85mm diameter silica-based coherent fiber bundle to an Olympus Fluoview 1000 confocal microscope. The pinhole was set to sample at one Airy unit and framesize set to image at Nyquist. AO and SR101 were imaged sequentially with 488nm and 560nm excitation, respectively. Imaging began at the brain surface and stepwise with 1mm increments to a depth of 5mm. Images were processed with linear functions utilizing NIH ImageJ.

Summary and Conclusions

In vivo imaging with SR101 provides rapid and specific identification of human astrocytic tumors in rodent orthotopic xenographs. SR101 is a robust live-cell imaging physiologic fluorophore routinely utilized to specifically identify astrocytic cells in live tissue. SR101's strong specificity to astrocytoma cells in fresh tissue has been previously reported. Coupled with a minimally invasive fluorescent microendoscope, we show SR101 may have utility for providing immediate biopsy-free *in vivo* histopathological diagnoses.

References

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