Handheld Spectroscopic Pen Device for Intraoperative Detection of GBM Fluorescence After 5-ALA Administration

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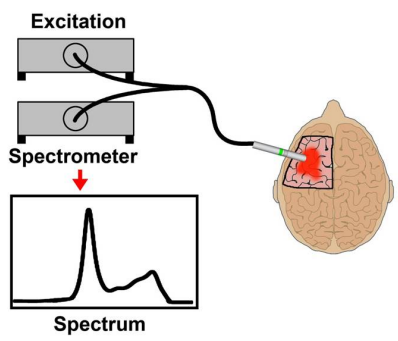
Introduction

Fluorescent-guided surgery (FGS) after 5-aminolevulinic acid (5-ALA) administration has been shown to result in more complete resection of malignant gliomas. Blue light (410 nm) emitted from the intraoperative microscope permits gross intraoperative visualization of protoporphorin IX (PpIX), metabolized from 5-ALA, in malignant glioma tissues. We have recently developed a hand-held spectroscopic pen device for intraoperative tumor detection based on wavelength-resolved measurements of fluorescent signals. Here, we present the use of this device for ultrasensitive GBM tumor cell detection in vitro and vivo after 5-ALA administration.



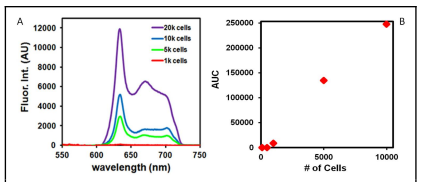
Schematic illustration showing hand-held spectrometer for brain tumor resection

Methods

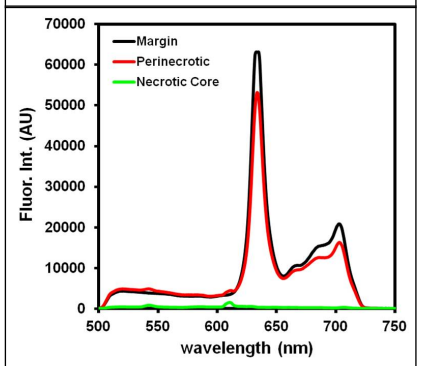
A spectroscopic device with violet LED excitation and a fiber optic probe was constructed. An in vitro study was conducted to test the sensitivity of this device with human GBM cells after 5-ALA treatment. Intracranial human GBM xenografts generated in rodents were also used to determine in vivo fluorescent measurements and sensitivity. Measurements were performed on xenografts and normal brain 2h after 5-ALA administration. Human GBM tumor specimens were also collected from newly diagnosed human patients enrolled in a Phase II 5-ALA study undergoing FGS of their tumor. Specimens were collected from 4 different regions of the tumor corresponding to the tumor bulk, necrosis, perinecrosis, and tumor margin. The specimens were analyzed ex vivo using the device to analyze PpIX signal.

Results

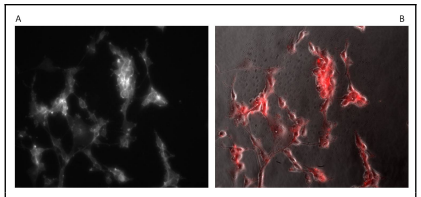
Sensitivity studies showed that the handheld pen device is capable of detecting <1000 tumor cells. Measurements in human specimens showed intense fluorescent signal from the tumor margin, bulk and perinecrotic regions while weak signal was found in the necrotic core. Analysis showed a >40:1 signal:noise ratio.



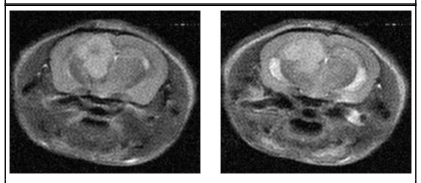
A. Sensitivity of the hand-held spectrometer to fluorescently labeled cancer cells. B. Protoporphyrin IX fluorescence spectra from cells incubated with 5-ALA for 24 hours Using the hand-held spectroscopic device, specimens containing as few as 1000 cells can be identified



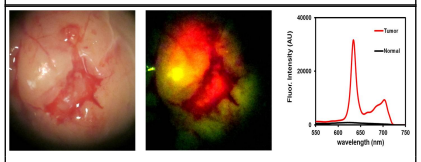
Ex vivo analysis of human tumor specimens from patients undergoing glioblastoma resection. Patients received 20 mg/kg 5-ALA 3-4 hours prior to surgery for fluorescence guided resection. Measurements from the hand-held spectroscopic device showed strong protoporphyrin IX signal in tissues identified as tumor positive.



U87MGEGFRvIII in vitro fluorescence images. A.Black&White image of the PPIX fluorescence signal. B.Merged image with the brightfield showing the PPIX fluorescence signal



T2-weighted MRI of a mouse brain showing a U87MGEGFRvIII xenograft



A.Brain of a mouse with a U87MGEGFRvIII xenograft 2 hours after intraperitoneal 5-ALA administration. B.Using the hand-held spectroscopic device, the tumor exhibited strong protoporphyrin IX fluorescence. C.The tumor can spectroscopically distinguished from normal brain, which showed no protoporphyrin IX fluorescence.

Conclusions

These studies demonstrate the potential of the device for intraoperative differentiation of tumor and normal tissue that is not possible with visual observation with the modified intraoperative microscope. Future work includes continued refinement of the device and a clinical trial for intraoperative guidance in glioblastoma resection.

Learning Objectives

1.Understand the use of 5-ALA and fluorescent-guided surgery for more complete resection of gliomas.
2.Introduction of hand-held spectroscopy device for PpIX fluorescence detection.
3. Use of hand-held device for detection of <1000 GBM cells and orthotopic human GBM xenografts.
4. Use of hand-held device for intraoperative fluorescence of different regions of GBM tumor.

References

Hadjipanayis CG et al. "Current and Future Clinical Applications for Optical Imaging of Cancer: From Intraoperative Surgical Guidance to Cancer Screening", Semin Oncol. 2011 Feb;38(1):109-18.
Roberts DW et al. "Glioblastoma multiforme treatment with clinical trials for surgical resection (aminolevulinic acid)",Neurosurg Clin N Am. 2012 Jul;23(3):371-7.