Detection of Radiation-Induced Brain Necrosis in Live Rats Using Label-free Fluorescence Lifetime Spectroscopy

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Introduction

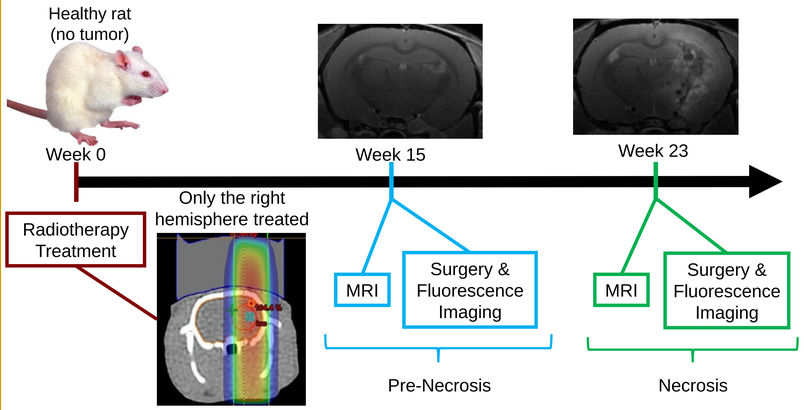
Background: Brain cancer is estimated to be diagnosed in 22,850 people in the US next year. In addition to surgical resection and chemotherapy, standard treatments for brain cancer include radiotherapy. This treatment, however, often kills healthy cells adjacent to the tumor, leading to undesirable radiation-induced necrosis. Current clinical imaging and diagnostic techniques cannot reliably differentiate between radiation induced necrosis and recurrent tumor in the brain. Fluorescence lifetime spectroscopy has previously been demonstrated to be able to discriminate tumor from healthy brain tissue [1] and represents a potential solution to this problem.

Hypothesis: Label-free fluorescence lifetime spectroscopy can be used to detect radiation-induced brain necrosis.

Goals: This study aims to (1) characterize and understand the fluorophore composition of necrotic and pre-necrotic brain tissues using fluorescence lifetime spectroscopy and (2) assess the potential real-time system to detect these changes in composition intraoperatively in live rats.

Methods

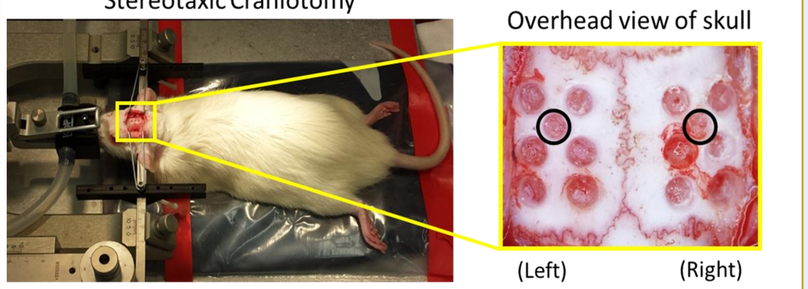
Radiotherapy Treatment: Healthy Fischer 344 rats received a single fraction 60 Gy radiotherapy treatment to the right hemisphere of the brain, using a Varian TrueBeam linear accelerator. The animals had T2-weighted preoperative scans to confirm the presence, or lack thereof, of necrosis.

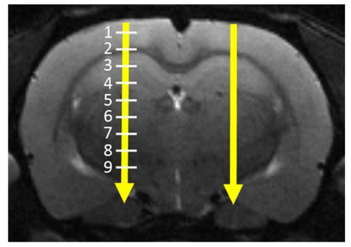


Surgery: All animals received terminal surgeries with fourteen burr holes to access the tissue. The pre-necrosis rodents underwent surgery at week 15, before necrosis was observed. Necrosis rodents underwent surgery at week 23. After data collection, animals were perfused and re-imaged with MRI for coregistration of the needle tracks with the preoperative scans. Histological analysis was performed with H & E staining.

Methods

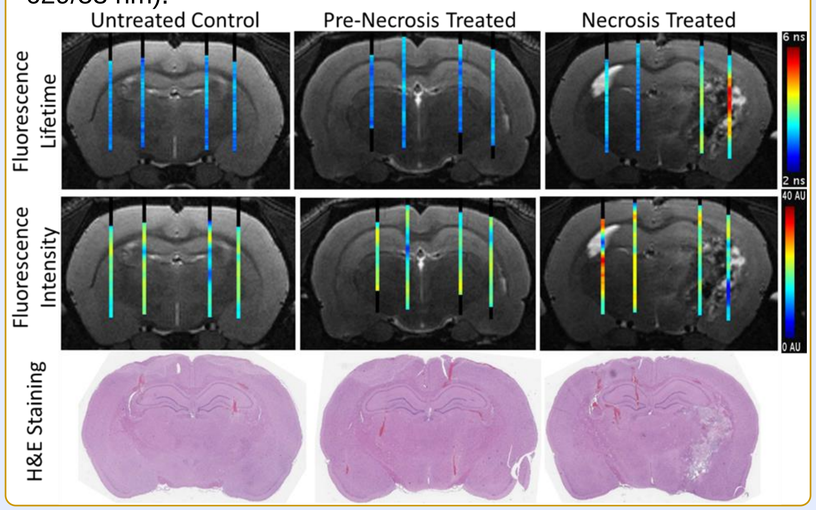
Time Resolved Fluorescence Spectroscopy (TRFS): Lifetime spectra of the brain tissue were recorded using a 355 nm pulsed laser for excitation, with a 600 um fiber optic. The scans were taken in the middle of the treated region on the right side and on the contralateral side, shown with black circles in the picture below. The fiber was inserted at an increment of 1 mm, until the bottom of the brain is reached (~ 9 mm).





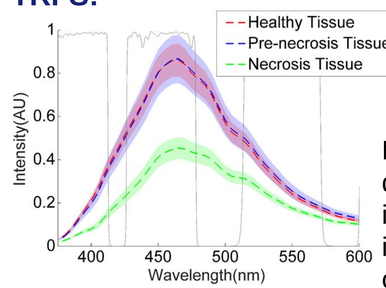
9 TRFS measurements taken at increasing depths (1 mm) from both left and right side of the brain

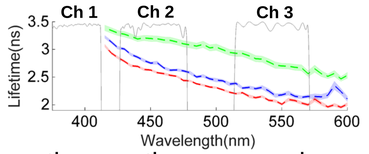
Real-time Multispectral Time Resolved Fluorescence Spectroscopy (msTRFS): Fluorescence data were also acquired over a depth scan of 9 mm in the remaining 12 burr holes, using a motorized 400 um fiber optic. The fluorescence emission was separated into four different wavelength channels (center wavelength/bandwidth: 390/40, 452/45, 542/50 and 629/53 nm).



Results

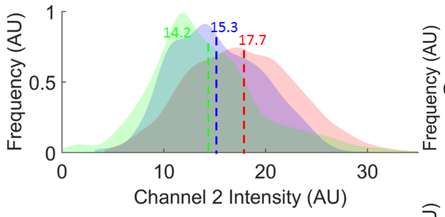
TRFS:

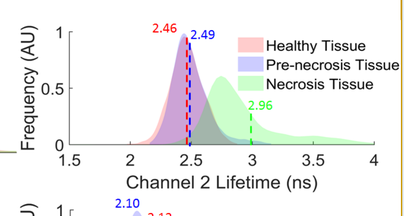


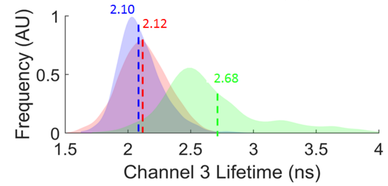


Necrotic tissue showed decreased intensity and increased lifetime. Small changes in lifetime, but not intensity were observed for pre-necrosis.

Real-time msTRFS:

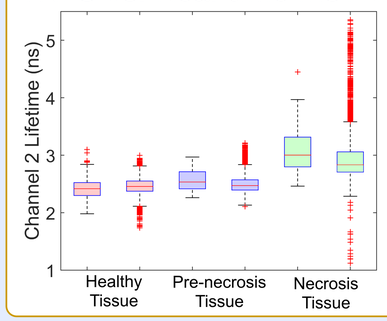






Results from ms-TRFS demonstrate large differences in fluorescence data between necrosis and healthy tissue.

TRFS vs. Real-time msTRFS:



The left and right box plots are calculations based on TRFS and the direct measurements with real-time msTRFS. Comparing the results between the two systems for each tissue type, the fluorescence lifetimes agree.

Conclusions:

These results show for the first time that radiation-induced brain necrosis tissue contains significantly different metabolic signatures that are detectable with label-free fluorescence lifetime techniques. Furthermore, it has provided critical guidance for an upcoming clinical trial to evaluate this method in patients.

References and Acknowledgements

1. L. Marcu, et al., “Fluorescence lifetime spectroscopy and imaging in neurosurgery,” IEEE Journal of Selected Topics in Quantum Electronics **18**, 1465-1477 (2012).

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