Real-Time Monitoring of Glutathione Dynamics In Lung Cancer Cells Following Radiotherapy Using Real Thiol–Based Optical Sensing and Fluorescence Microscopy

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ABSTRACT

Glutathione (GSH) is a key intracellular antioxidant that regulates cellular redox homeostasis and plays a central role in determining tumor response to radiotherapy. However, real-time monitoring of radiation-induced GSH dynamics remains challenging. We developed a multimodal platform to quantify GSH dynamics in A549 lung cancer cells using the reversible ratiometric probe RealThiol AM. Probe performance was calibrated against known GSH concentrations, and radiation-induced GSH changes were evaluated in two-dimensional monolayers and three-dimensional tumor spheroids following 10 Gy X-ray irradiation using fluorescence microscopy, flow cytometry, and a custom optical fiber coupled spectroscopic sensor. All three platforms consistently detected radiation induced GSH depletion, demonstrating good agreement between imaging, cytometric, and spectroscopic measurements. These results establish a robust platform for quantitative, real-time monitoring of intracellular GSH during and after radiotherapy and provide the foundation for ongoing studies comparing redox responses following conventional and FLASH irradiation.

MATERIALS AND METHODS

- **Cell model:** A549 lung cancer cells cultured as 2D monolayers and 3D tumor spheroids.
- **GSH probe:** Intracellular GSH measured using RealThiol AM (RT-AM), a ratiometric fluorescent probe (405 and 488 nm excitation).
- **Radiation studies:** Cells irradiated with **10 Gy X-rays**, followed by time resolved fluorescence microscopy to monitor GSH dynamics.
- **Flow cytometry:** Used to independently quantify radiation induced changes in intracellular GSH.
- **Optical fiber sensor:** A custom fiber optic platform was developed and calibrated for real-time ratiometric GSH measurements in 3D tumor spheroids.

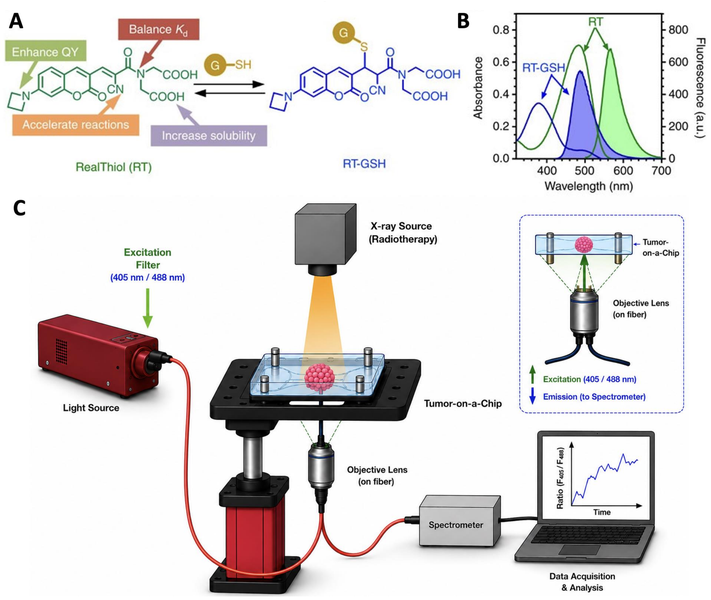
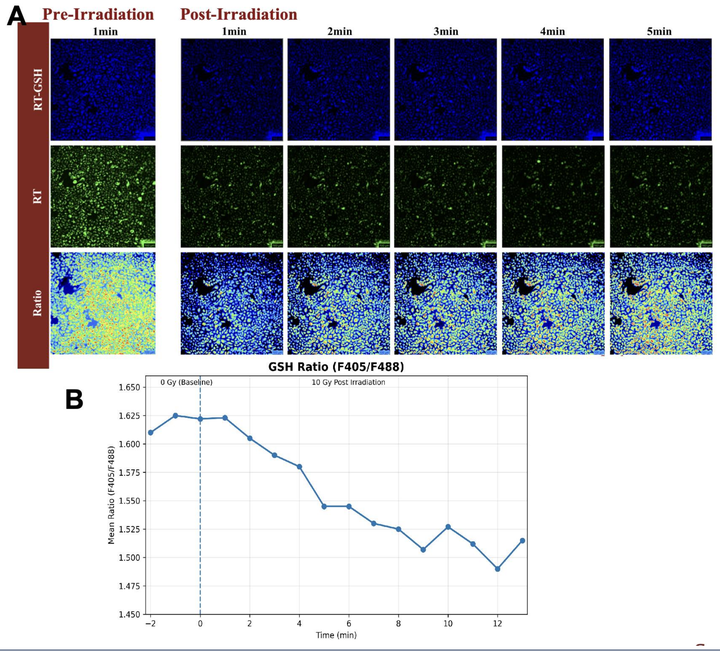


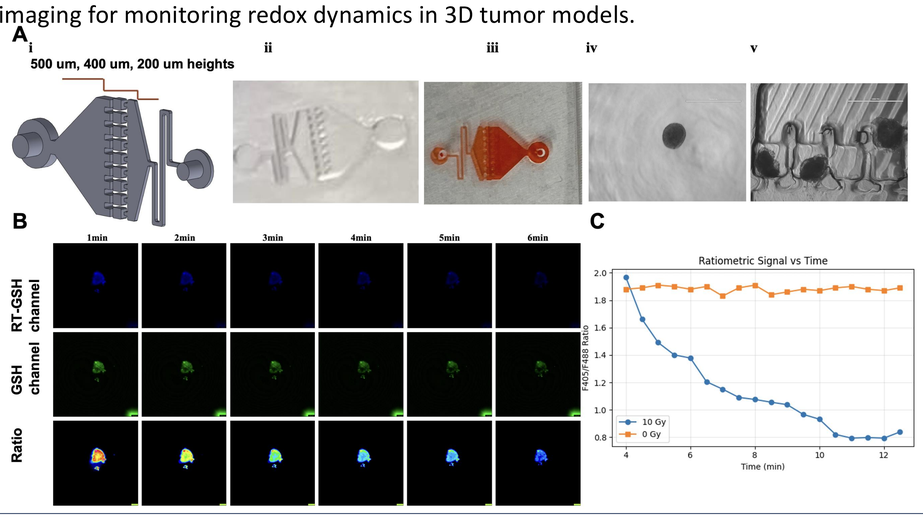
Figure 1. Overview of the optical fiber-based glutathione sensing platform for real time monitoring of intracellular GSH during radiotherapy.

RESULTS

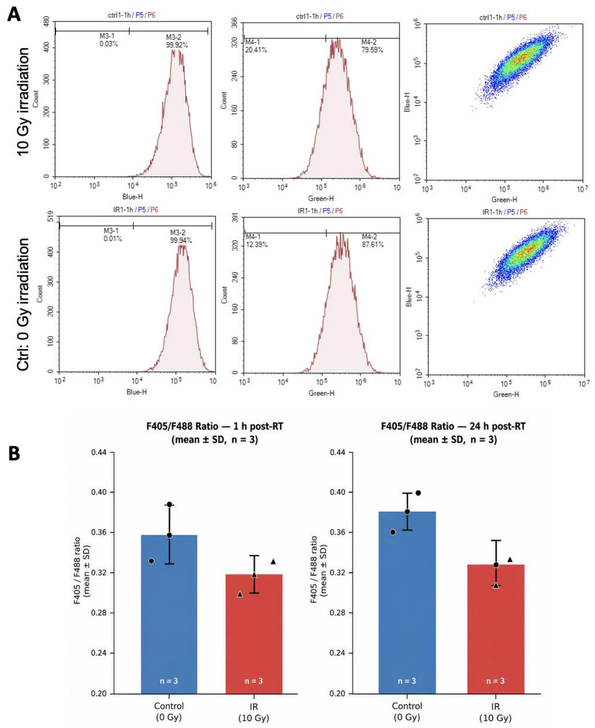
Result 1: Radiation-induced GSH depletion was visualized in A549 tumor spheroids using RealThiol-based fluorescence microscopy. Representative RT, RT-GSH, and ratiometric fluorescence images are shown in **(A)**. Quantitative analysis of the ratiometric fluorescence signal confirmed radiation induced intracellular GSH depletion in A549 tumor spheroids compared with nonirradiated controls **(B)**.



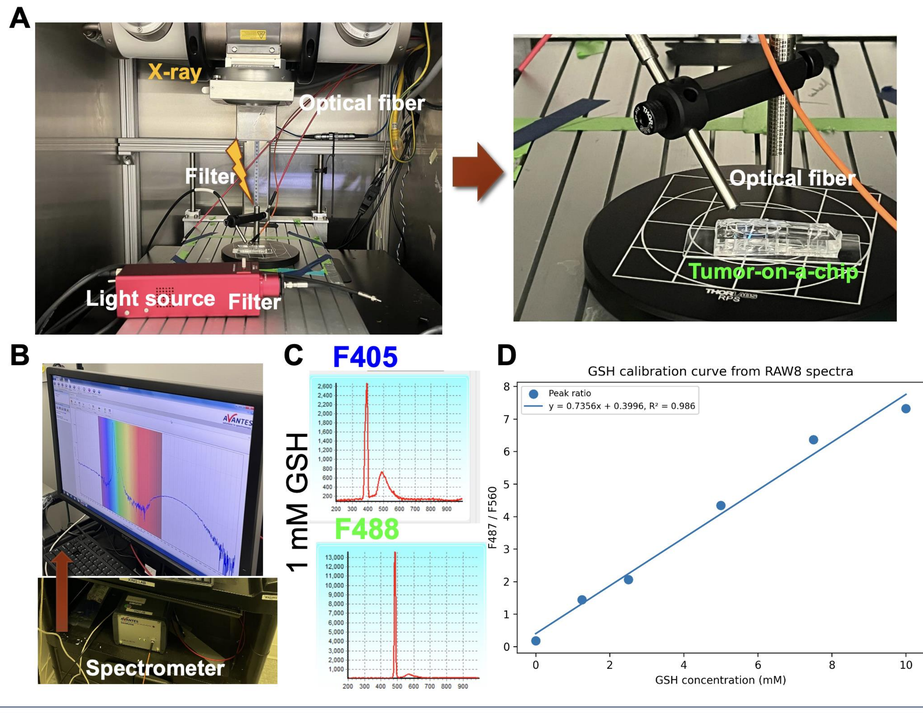
Result 2: A microfluidic tumor-on-a-chip platform was developed for real-time monitoring of radiation-induced GSH dynamics. The chip fabrication workflow and integration of A549 tumor spheroids are shown in **(A)**. Representative RT, RT-GSH, and ratiometric fluorescence images following 10 Gy X-ray irradiation are presented in **(B)**. Quantitative analysis confirmed radiation-induced GSH depletion compared with nonirradiated controls **(C)**, demonstrating the feasibility of RealThiol-based ratiometric imaging for monitoring redox dynamics in 3D tumor models.



Result 3: Flow cytometry independently validated radiation induced GSH dynamics in A549 cells. Representative fluorescence histograms and Blue (RT-GSH) versus Green (RT) density plots for irradiated and nonirradiated cells, acquired 1 hour after irradiation, are shown in **(A)**. Quantitative analysis of the RealThiol ratiometric fluorescence signal (F405/F488) demonstrated GSH depletion at 1 hour followed by partial recovery at 24 hours after irradiation **(B)**, consistent with the fluorescence microscopy measurements.



Result 4: A custom optical fiber-based sensing platform was developed for real-time monitoring of intracellular GSH during radiotherapy. The experimental setup integrated within the X-ray irradiator cabinet and the fiber coupled spectrometer are shown in **(A)** and **(B)**. Representative fluorescence spectra acquired following 405 nm and 488 nm excitation are presented in **(C)**. The optical sensor demonstrated a linear relationship between the RealThiol ratiometric fluorescence signal (F405/F488) and reduced GSH concentration **(D)**, supporting its feasibility for quantitative real-time GSH sensing.



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CONCLUSIONS

Real thiol-based ratiometric GSH sensing enables quantitative assessment of radiation-induced intracellular GSH dynamics in tumor models. Fluorescence microscopy, flow cytometry, and optical fiber sensing provided complementary validation of radiation-induced GSH depletion, while the optical platform establishes the basis for continuous real-time monitoring during and after irradiation. This work provides a foundation for ongoing studies comparing redox responses following conventional and FLASH radiotherapy and supports mechanistic investigations.

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