Metabolic Imaging of Human Breast Carcinoma Treatments

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Abstract

Two-photon fluorescence imaging is capable of detecting changes in cellular metabolism by quantifying the autofluorescent metabolic co-enzymes NADH and FAD. Two-photon microscopy measures their fluorescence lifetimes and relative intensities (redox ratio). However, these measurements neglect the possible effects of glucose uptake and mild hyperthermia in breast cancer cells. Cells were incubated with the fluorescent glucose molecule 2-NBDG and imaged using two-photon microscopy. Additionally, cells were treated with anticancer drugs to investigate their effects on 2-NBDG uptake. The findings demonstrated a significant ($p<0.05$) decrease in 2-NBDG fluorescence in treated cells versus untreated cells. Furthermore, treated and untreated breast cancer cells were incubated with iron oxide-coated gold nanorods and imaged to determine whether nanorod presence altered cellular metabolism. Controlled photothermal heating of nanorods in distilled water verified their heating capability, yet two-photon imaging indicated that the nanorods were not functionalized properly for i*n vitro* applications.

Hypotheses

- Breast cancer cells incubated with 2-NBDG will have increased fluorescence intensity versus controls. Cancer therapeutics used here will decrease 2-NBDG uptake in cell lines responsive to such therapeutics.
- The presence of gold nanoparticles alone will not have any effect on glucose uptake and cell metabolism. When gold nanoparticles are used in conjunction with low photothermal heat generation, glucose uptake will be altered.

Materials & Methods

Breast Cell Lines

	ER	PR	HER2
BT474	+	+	+
MCF7	+	+	-
MDA-MB-231	-	-	-

Table 1. Human breast cancer cell lines used in experiments with 2-NBDG and nanorods. Indicates ER/PR positivity and HER2 overexpression

Therapeutics

Drug	Action
XL147	PI3K Inhibitor
Trastuzumab (Herceptin)	HER2 Inhibitor
Paclitaxel	Chemotherapy

Table 2. Examples of drugs to be tested which have shown promise in treating drug-resistance in human breast tumors

Gold Nanorods & Photothermal Heating

Mild Hyperthermia → Tumor Reoxygenation → Improved Patient Treatment Response

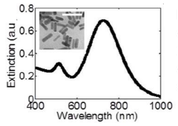
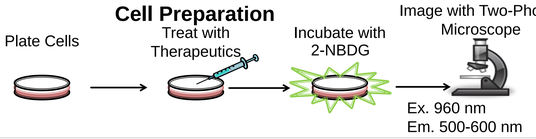


Figure 1. Representative image of gold nanorods (scale bar = 100 nm) and corresponding emission spectrum with peak at approximately 740 nm.

Cell Preparation



Cell Profiler

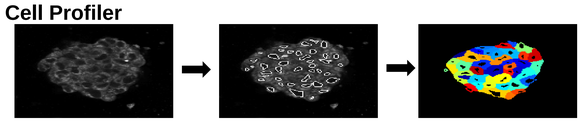


Figure 2. An automated image analysis method was created using CellProfiler and Matlab (www.cellprofiler.org) in order to segment individual cell cytoplasms in a monolayer or organoid image. This allows for the detailed analysis of subpopulations of cells which may contribute to tumor drug resistance [5].

2-NBDG Uptake

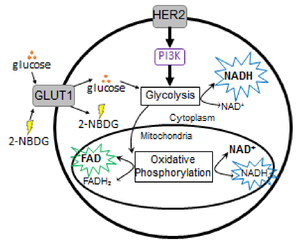


Figure 3. A model of a cancer cell illustrating the process of 2-NBDG uptake at the GLUT1 receptor through cellular glycolysis.

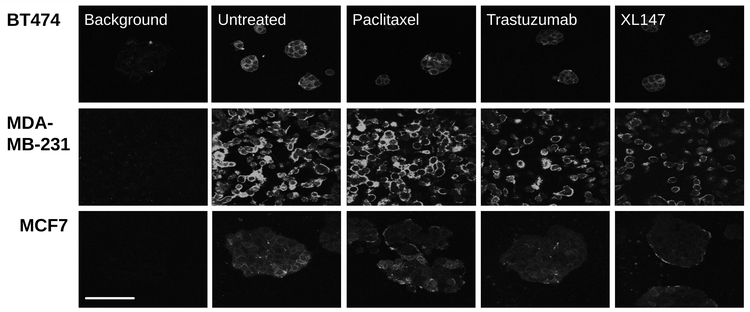


Figure 4. 2-NBDG fluorescence images taken with two-photon microscopy. Scale bar = 100 μm

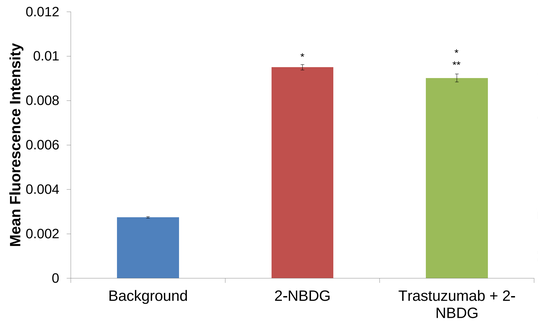


Figure 5. Mean fluorescence intensity increases in BT474 cells when incubated with 2-NBDG compared to non-glucose DMEM media. Treatment with trastuzumab decreases the 2-NBDG fluorescence of BT474 cells compared to untreated cells. *=($p<0.05$ vs. Background) **=($p<0.05$ vs. 2-NBDG)

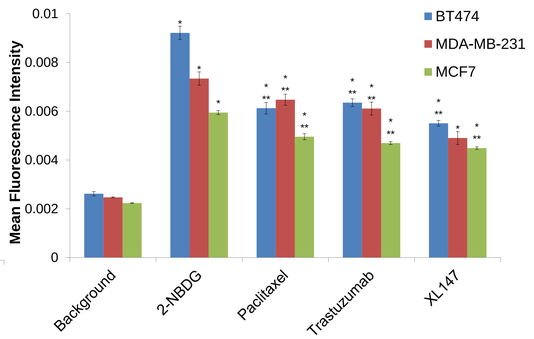


Figure 6. Mean fluorescence intensity increases in all three cell types following 2-NBDG incubation. Cancer therapeutic drugs decreased the fluorescence intensity in all cases. *=($p<0.05$ vs. Background) **=($p<0.05$ vs. 2-NBDG)

Gold Nanorods & Photothermal Heating

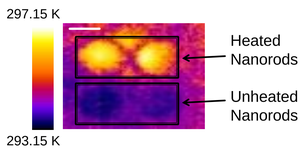


Figure 7. Iron-oxide coated gold nanorods are heated at 200 mW using a continuous wave 785 nm laser and imaged using a FLIR thermal camera. Scale bar = 5 mm

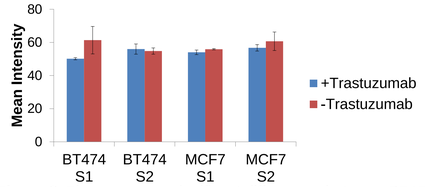


Figure 8. There were no significant differences between BT474 and MCF7 cells when incubated with iron-oxide gold nanorods. This is likely due to improper functionalization.

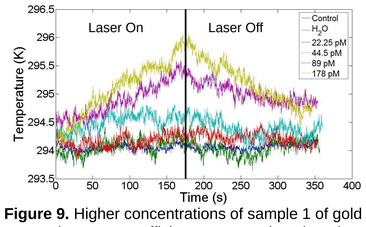


Figure 9. Higher concentrations of sample 1 of gold nanorods are more efficient at generating photothermal heat than lower concentrations.

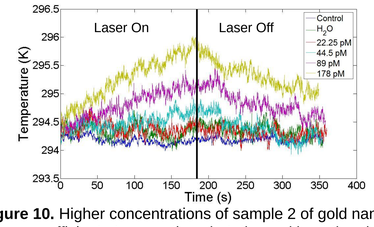


Figure 10. Higher concentrations of sample 2 of gold nanorods are more efficient at generating photothermal heat than lower concentrations.

Conclusions

- Mean fluorescence intensity from two-photon microscopy increases in BT474, MDA-MB-231, and MCF7 breast cancer cells following 2-NBDG incubation.
- Cancer therapeutics decrease the 2-NBDG fluorescence intensity in all cell types, thus decreasing glucose uptake.
- 2-NBDG fluorescence imaging is a successful method for measuring glucose uptake in human breast carcinoma cells.
- 2-NBDG uptake as a biological marker has the potential to improve the quantification of treatment response in breast cancer.
- Iron oxide-coated gold nanorods are effective in generating photothermal heat and have the potential to be used in mild hyperthermia treatment.
- More work must be done to properly functionalize the gold nanorods for *in vitro* and *in vivo* applications.

Future Directions

- Further work will be done to properly functionalize the iron oxide-coated gold nanorods for use *in vitro*. Once the nanorods are functionalized appropriately, fluorescence imaging will measure the effect of the nanorods on cellular metabolism.
- 2-NBDG fluorescence imaging will be expanded to three-dimensional organoids with the eventual goal of using fluorescence measurements to determine optimal treatment for breast cancer patients on an individual basis.

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