Bioluminescent Metabolite Assays Enable Easy Measurement of Changes in Tumor Biology

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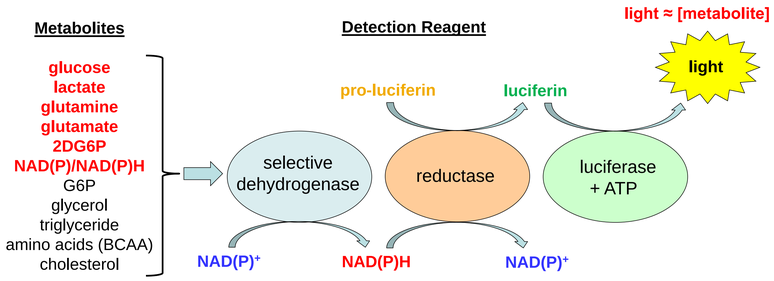
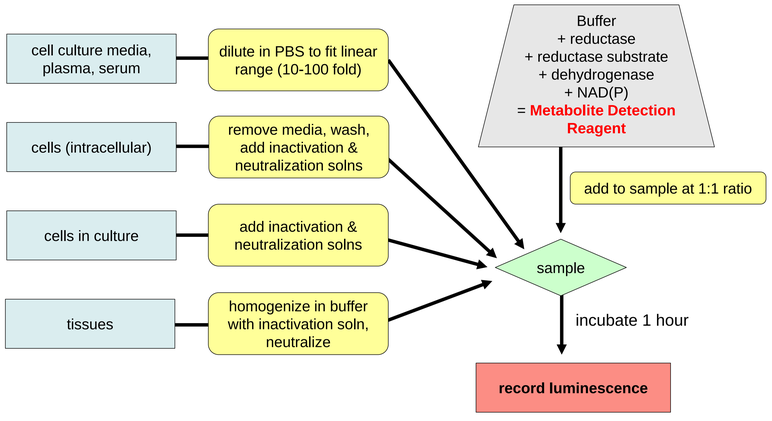
Abstract # 4026

1. Introduction

Tumor cells rely on metabolic reprogramming to meet their increased demands for energy and biomass. Such reprogramming is exemplified by increased rates of glucose uptake, greater utilization of glycolysis, and a reliance on glutaminolysis. We have developed a suite of bioluminescent assays that can monitor metabolites in all of these important pathways.

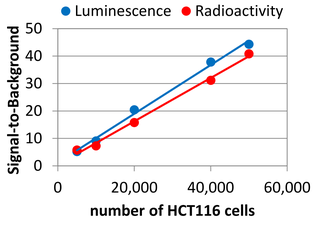


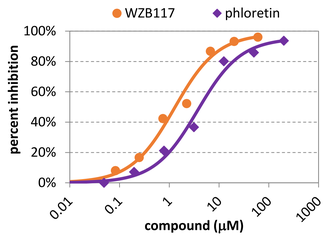
2. Protocols for Different Sample Types



3. Glucose Uptake Assay

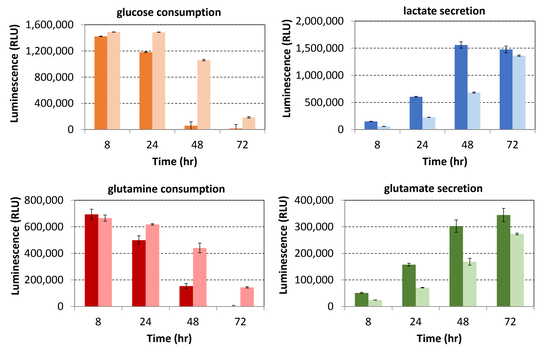
To measure glucose uptake, a glucose analog (2-deoxyglucose, 2DG) is added to live cells, it is transported inside the cell, and it is then phosphorylated to form an impermeable metabolite (2-deoxyglucose-6-phosphate, 2DG6P) that is not further metabolized. The accumulated 2DG6P is detected with glucose-6-phosphate dehydrogenase. The assay performs comparably to the radioactive assay (left graph) and correctly measures inhibition of glucose transporters (right graph).





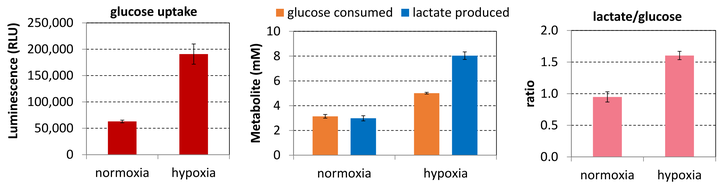
4. Monitoring Changes in Metabolites Over Time

A549 cells were plated at 15,000 (dark bars) or 5,000 (light bars) cells per well in a 96 well plate. At the indicated time points, 2.5 ul aliquots were removed, diluted in PBS, and frozen for later analysis.



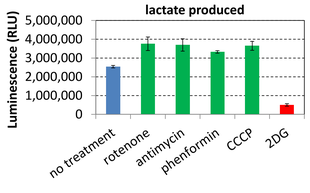
5. Hypoxia Induced Changes in Metabolism

MCF7 cells were grown under normoxic or hypoxic (1% oxygen) conditions for 24 hours prior to assay. By depleting oxygen, hypoxia shifts the metabolic program from oxidative phosphorylation towards glycolysis, which results in an increase in glucose uptake and an increase in the amount of lactate produced vs glucose consumed.

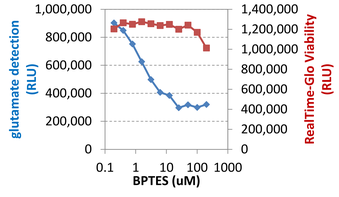


6. Effectors of Glycolysis and Glutaminolysis

A549 cells were plated at 15,000 cells per well in a 96 well plate and incubated for 1 hour with a variety of compounds before measuring lactate production. Mitochondrial inhibitors (5µM rotenone, 5µM antimycin, 2.5mM phenformin, or 50µM CCCP) slightly increase lactate, whereas the glycolytic inhibitor 2DG (10 mM) significantly decreases lactate.

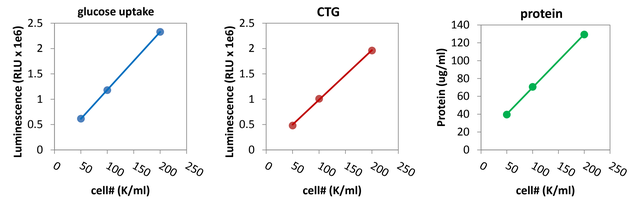


SKOV-3 cells were plated at 4,000 cells per well in a 384 well plate and incubated for 1 hour with medium containing RealTime-Glo™ Cell Viability Assay Reagent (RTG) and a titration of the glutaminase inhibitor bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES). The signal from RTG was measured just prior to measuring glutamate.



7. Assay Multiplexing

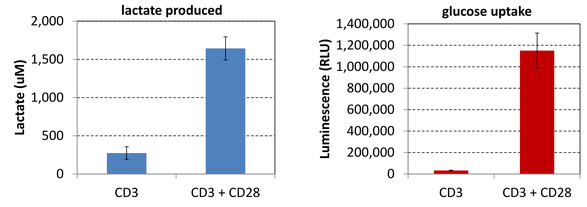
The glucose uptake assay was applied to a titration of HCT116 cells. After inactivation, aliquots were removed to measure viability (CellTiter-Glo, CTG) and protein concentration (Pierce 660 nm Protein Assay Reagent + IDCR).



Other multiplexing options for measuring viability include addition of RealTime-Glo™ (see panel 6) or CellTiter-Fluor (data not shown) Cell Viability Assays to the media for 1 hour. Viability is measured just prior to metabolite detection.

8. Increased Glycolysis in Activated T Cells

Primary human CD4+ T cells were incubated with antibody-conjugated beads containing anti-CD3 +/- anti-CD28 and plated at 250,000 cells per well in a 96 well plate. After 24 hours, medium was removed to measure lactate production, the cells were washed to remove glucose, and glucose uptake was measured. The signal reported from the glucose uptake assay is the net luminescence after subtracting a negative control (i.e. pre-incubation with 50 µM cytochalasin B, a glucose transporter inhibitor).



9. Conclusions

- **Bioluminescent metabolite assays can facilitate the study of cellular energy metabolism**
  - Assays for measuring key metabolites can provide useful information for studies of glycolysis and glutaminolysis
  - Radioactivity is not needed for glucose uptake measurements
  - Assays are done in multiwell plates and are suitable for higher-throughput formats
  - Multiplexing can provide more information per well and facilitates data normalization
- **Bioluminescent metabolite assay features**
  - Flexibility: assay different sample types with minimal sample preparation
  - Sensitivity: use small amount of sample and low numbers of cells per well (e.g. 1000 cells/well)
  - Broad linearity: measure metabolite concentrations over 2 to 3 logs
  - Wide assay windows: discriminate small changes due to large S/B

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