Bioluminescent Metabolite Detection Assays for Investigating Metabolic Pathways
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1. Introduction
Nutrient consumption, metabolism, and energy production are connected, regulated processes. The rewiring of these processes in cancer cells allows abnormal growth and adaptation to the tumor microenvironment. Increased interest in tumor metabolism has generated a need for assays that have higher throughput to facilitate rapid testing of several samples. We developed bioluminescent plate-based detection assays for measuring the metabolic differences between cells and metabolic changes in response to small molecule compounds and altered environmental conditions.

2. Protocol and Sample Preparation
Assays Detect Metabolites in a Variety of Samples with Minimal Sample Preparation
- Sample types include cell lysates, cell culture medium, tissues and plasma
- Sample preparation depends on sample type and may include dilution in buffer
- With the proper selective dehydrogenase, the technology can be extended to other metabolites such as branched chain amino acids
- MetaboLite Assays use selective dehydrogenases to produce NAD(P)H in proportion to the amount of metabolite present
- NAD(P)H is detected using a Reductase enzyme and its Proluciferin substrate in combination with a luciferase enzyme to produce light

3. Monitoring Nutrient Consumption and Metabolite Secretion
Monitoring metabolites in cell culture medium can provide information about cellular metabolic pathways. For example, glucose consumption and lactate secretion can serve as indicators of glycolysis, while glutamine consumption and glutamate secretion can provide information about glutaminolysis. By sampling small volumes of medium, changes can be monitored online or after treatments, such as exposure to hypoxic conditions.

4. Homogeneous Assay for Effectors of Glucose Metabolism
Effectors of glucose metabolism can be identified by using lactate as a glycolytic indicator. To rapidly detect changes in cellular lactate production, we developed an in-vitro homogeneous assay format. Using this format, plated cells were incubated with compounds for 1hr before adding acidic lysis solution and lactate detection reagent to the wells. This format does not require the removal of medium or the washing of cells, and can facilitate the screening of both inhibitors and activators of glycolysis.

5. Homogeneous Assay for Effectors of Glutamine Metabolism
Analogous to using lactate production as a marker for changes in glycolysis, glutamate production can provide insights regarding glutaminolysis. The homogeneous glutamate assay was validated using a well known glutaminase inhibitor and then used to screen the LOFAC® library (Sigma) for compounds that both decreased and increased glutamate production. Counter screens using glutamate controls and viability reagents were included to detect effects on the detection system or viability.

6. Metabolites in Tissues and Plasma
Metabolites can be measured in other sample types such as homogenized tissues or diluted plasma and sera. Only small amounts of sample are needed (e.g., 10mg tissue or 5µl plasma) and increased glutamate production. Counter screens using glutamate controls and viability reagents were included to detect effects on the detection system or viability.

7. Glucose Uptake Assay
The bioluminescent glucose uptake assay measures the uptake of the commonly used glucose analog, 2DG. Once in the cell, 2DG is phosphorylated forming 2DG6P, a stable, impermeable metabolite that is not further metabolized. Accumulated 2DG6P is detected using the selective dehydrogenase G6PDH.

Protocol
Cells after drug treatment
Glucose uptake by HCT116 cells

8. Immunometabolism: Activation of T Cells
Activation of T Cells Triggers Increase in Glucose as indicated by Increased Glucose Uptake and Lactate Secretion

9. Conclusions
Tumor metabolism is an important area of research and a target for the development of new therapeutics. In this poster we have described bioluminescent plate-based assays that can be used to facilitate the analysis of metabolic pathways and the screening of compound libraries.

Bioluminescent metabolite assays can facilitate the study of cellular energy metabolism
- Measurements of key metabolites can provide useful information during studies of glycolysis and glutaminolysis
- Assays are amenable to low volume multi-well plates, automation, and high-throughput formats
- Multiplexing capabilities with cell viability assays provides more information per well and facilitates data normalization

Bioluminescent metabolite assay benefits
- Sensitivity: small amount of sample and low numbers of cells per well (e.g., 1000 cells/well)
- Broadly: 2 to 3 logs provides convenience for testing samples at different metabolite concentrations
- Wide assay windows: S/B > 100 allows for better discrimination of small changes

References:

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