

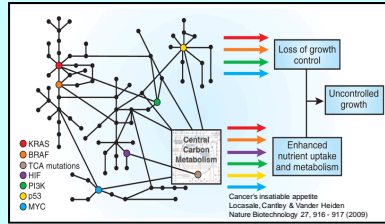
# A Mass Spectrometry Platform to Quantitatively Profile Cancer Cell Metabolism from Cell Lines to Tissues

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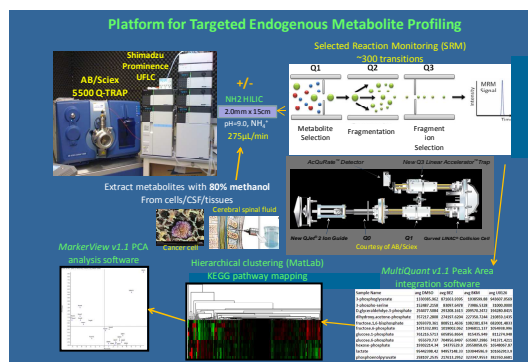
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We demonstrate the capabilities of a metabolomics profiling platform that we implemented to quantitatively target 250 endogenous water soluble cellular metabolites via SRM

It's more than just kinase activity and genetic defects in cancer



In order for tumor cells to grow and divide, defects in signaling pathways due to mutations, amplification, etc. need to translate to enhanced nutrient uptake and a loss of growth control.  
- Cell metabolism must be altered.



In order to study cancer cell metabolism, we developed a platform using the AB/Sciex 5500 QTRAP that targets 250 endogenous water soluble metabolites from 300 selected reaction monitoring SRM transitions during a single LC/MS/MS experiment and can be used with any cell or tissue source. We then integrate peak areas from Q3 TIC using both commercial and in-house developed clustering tools.

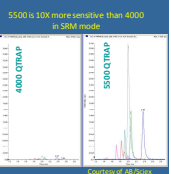
## Metabolomics Profiling Platform Details

Profile and target 250 endogenous water soluble cellular metabolites (300 SRM) covering pathways in glycolysis and metabolism  
-many SRM transitions based work developed by Josh Rabinowitz, Princeton Univ.

Robust and reproducible data from ~200 metabolites

Uniqueness of our platform:

Positive/negative ion switching within same 25 min. LC/MS/MS run  
• No ion switching time - Fast!  
• Far quicker than 4000 QTRAP

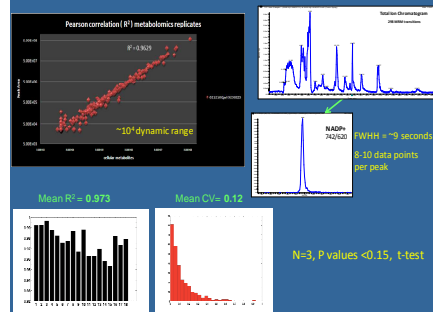


No chromatographic scheduling for SRM  
• List of 300 SRM transitions (2 sec. cycle time)  
• 5 ms SRM dwell time/can go to 2 ms if needed

Amino HILIC normal phase chromatography in both Positive and Negative mode.  
• Luna NH2 2.0 mm x 15 cm, Phenomenex

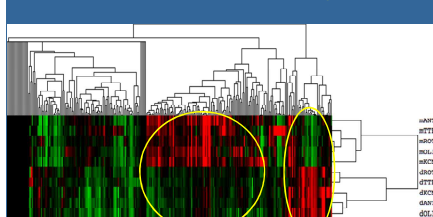
We accomplish this in a single run with a 2.0mm x 15cm amino hydrophilic interaction chromatography (HILIC) column at pH=9.0 using positive/negative ion switching. Since our cycle time is only 2.0 seconds, we do not use chromatographic scheduling for SRM. This results in robust and reliable data for ~200 metabolites.

## Robust and Reproducible



We show a 10<sup>4</sup> dynamic range, observe mean R<sup>2</sup> values of ~0.97 across replicates and coefficient of variation (CV) values of less than 0.15. Triplicate runs result in p values less than 0.15 and our platform acquires 8-10 data points per metabolite peak with a peak width of 9 seconds at FWHH.

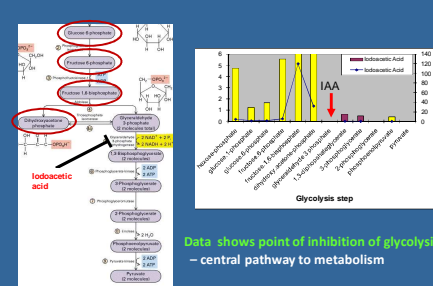
## Hierarchical Clustering of Human and Drosophila Cells Treated with 5 Anti-Metabolite Drugs



• Lots of metabolic effects  
• Shows vast differences between cell types

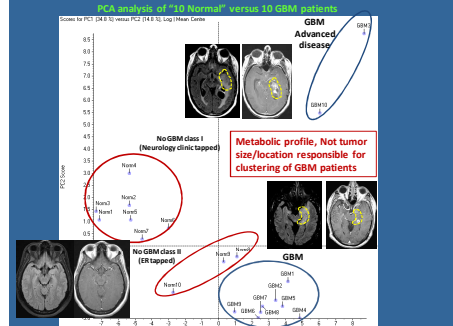
Hierarchical clustering is carried out on the integrated peak area lists across experimental conditions using both freeware (Cluster, dChip, etc.) and internally developed clustering tools programmed in MatLab. This example shows 5 anti-metabolite across a human and fly cell line.

## Metabolomics Platform Can Resolve Drug Targets in Metabolic Pathways - Glycolysis



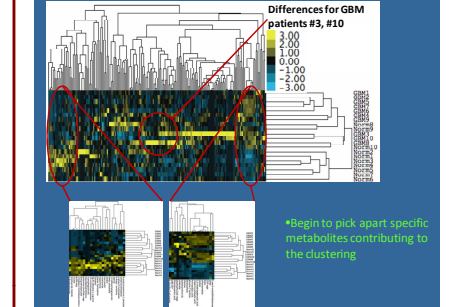
Our platform can resolve most metabolic members of the glycolysis pathway in addition to other major metabolic pathways. Idoic acid is a known inhibitor of glycolysis and we can determine the point of inhibition of glycolysis, here at the GAPDH step. The result is a buildup of glycolysis products up to dihydroxyacetone-phosphate and no production of glyceraldehyde-3-phosphate or any metabolites downstream in the pathway.

## Direct Clinical Application: Metabolomics Profiling in Cerebral Spinal Fluid (CSF) of Glioblastoma Patients at BIDMC



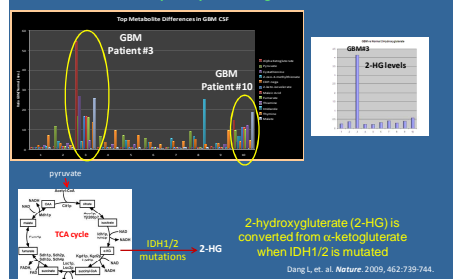
As a clinical test, the cerebral spinal fluid (CSF) from 20 patients were profiled using our metabolomics platform (10 normal and 10 with glioblastoma (GBM)). The platform was capable of clustering the normal patients from the GBM patients using principal components analysis (PCA). For normal and GBM, two clusters were identified and only the metabolomics profile, not MRI scans could distinguish these GBM patients as tumor size and location were similar.

## Hierarchical Clustering of CSF from 20 BIDMC Patients



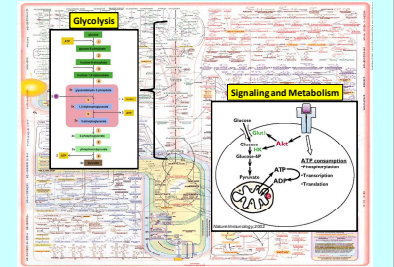
Hierarchical clustering of the 20 patients can help distinguish which metabolites contribute to the PCA clustering groups. Notice that groups of metabolites are different between normal and GBM patients.

## Metabolite outliers from GBM patients over average of normal pool by fold change

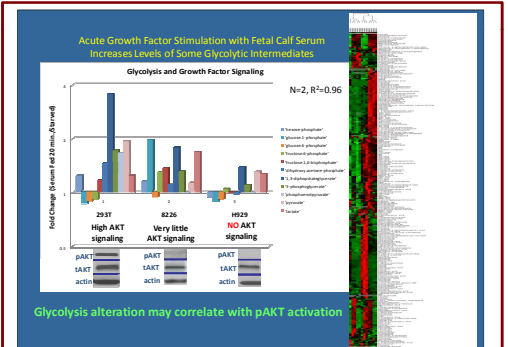


Some of the metabolites contributing to the cluster of GBM patients #3 and #10 are members of the TCA (Krebs) cycle. Interestingly, GBM patient #3 shows high levels of 2-hydroxyglutarate (2-HG), a metabolite produced from a mutation of IDH1/2 and prevalent in glioblastoma.

## Metabolic Pathway (Subway) Map

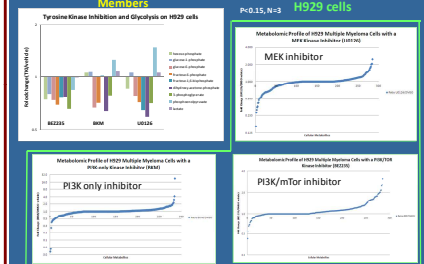


Metabolic pathways are complex but we can focus on the few central and ancient pathways such as glycolysis for an initial screen of cellular response. In addition, metabolism and cell signaling work in synergy to grow and divide cells.

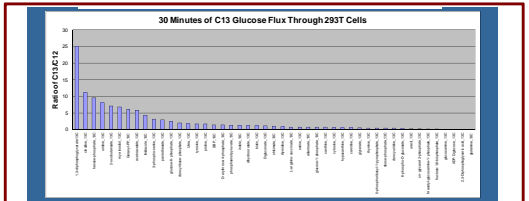


Analysis of the glycolysis pathway reveals that increased levels of glycolytic intermediates may correlate directly with the level of cellular pAKT levels. For example, H929 multiple myeloma cells rarely exhibit pAKT activity while 293T cells signal through pAKT at high levels.

## Acute Tyrosine Kinase Inhibition Results in a Decrease of Glycolysis Pathway Members



One question we are asking is whether growth factor signaling (primarily AKT signaling) is directly affecting glycolysis on a short time scale including tyrosine kinase inhibition.



We quantitatively measure metabolic flux by treating cells with C13 labeled glucose and/or glutamine and measure the destination of labeled carbon atoms via SRM.

## SUMMARY

• A quantitative metabolomics platform was implemented using the 5500 QTRAP to profile 250 endogenous water soluble metabolites from a single 25 min. HILIC SRM experiment with pos./neg. switching