Metabolomics and lipidomics
New dimensions in understanding health and disease

Elizaveta (Lisa) Freinkman, Ph.D.
Research Scientist & Manager
Whitehead Institute Metabolite Profiling Core Facility

Whitehead Institute Teacher Program
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Outline and learning objectives

Small molecules and lipids are a key aspect of phenotype

Measuring small molecules and lipids is challenging because of their extre chemical diversity and wide concentration range

Liquid chromatography / mass spectrometry (LC/MS) is the single most broadly applicable technology in metabolomics

Metabolomics is delivering important insights to enable basic disease research, diagnostics, and drug development

Small molecules & lipids play a key role in biology

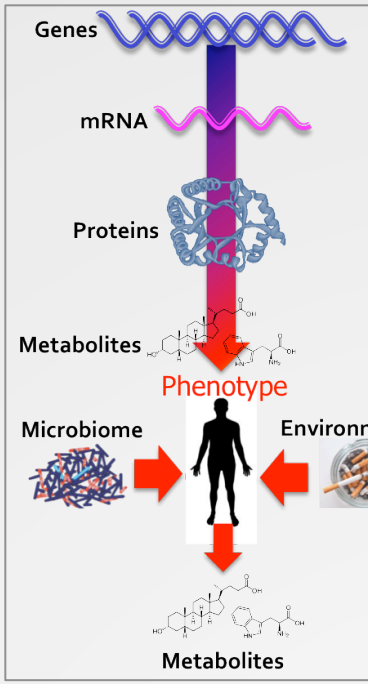
Nutrients – e.g., glucose, amino acids, vitamins

Building blocks – e.g., nucleotides, cholesterol

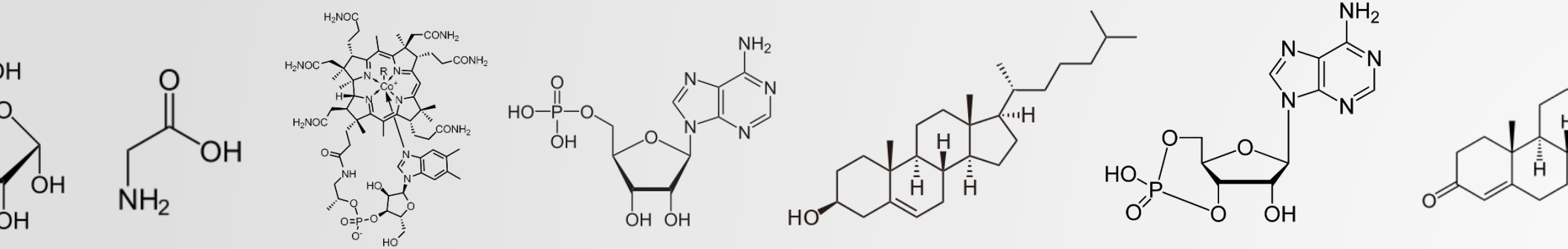
Signals – cAMP, steroid hormones

Metabolic enzymes and transporters make up ~2000 of the ~20000 genes in the human genome

Many other genes regulate and sense metabolite levels (without carrying out a chemical reaction)



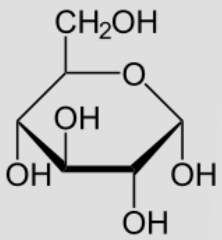
Shafizadeh T, *Metabonews*



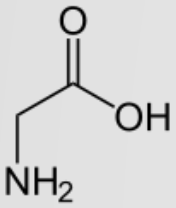
Challenges of small molecule & lipid analysis

- Extreme chemical diversity and specificity
  - Compare to DNA/RNA (4 building blocks) and proteins (20 building blocks)
- Wide concentration range
- Not directly encoded in genome
  - How many metabolites are there?? Estimates range from 2,000-30,000+

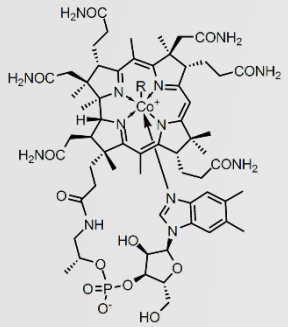
Glucose
~5 mM



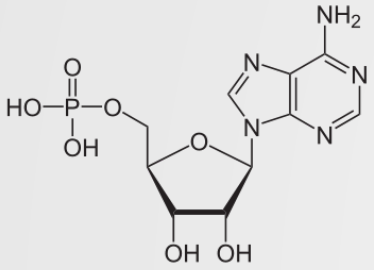
Glycine
MW = 78



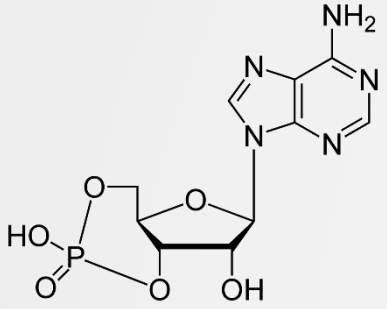
Vitamin B12
MW = 1,355



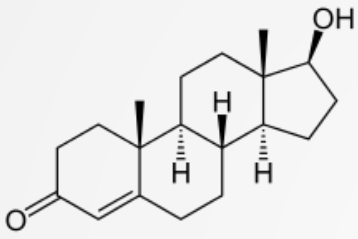
AMP



cAMP



Testosterone
~5 nM



How do we measure small molecules and lipids?

Enzyme- and antibody-based assays

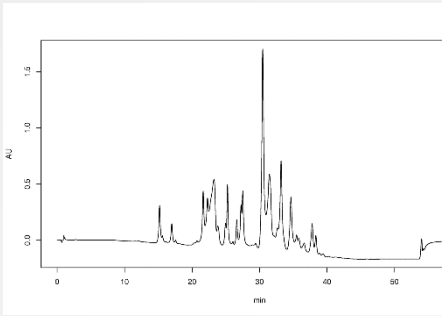
- Often quite sensitive, specific, and high-throughput
- Assay development takes a long time for each metabolite

Nuclear Magnetic Resonance (NMR)

- High specificity, low sensitivity (~70 metabolites)

Ultraviolet (UV) detection

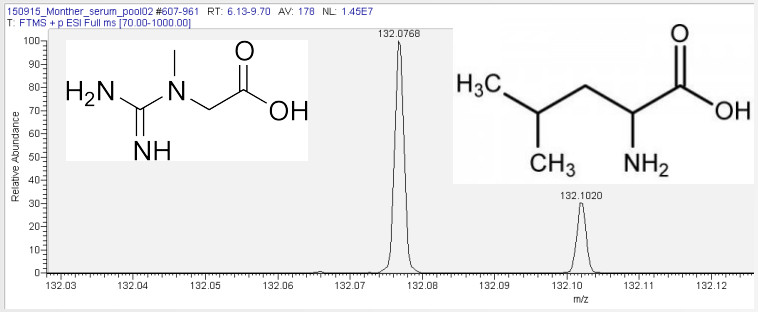
- Low specificity; requires extensive pre-fractionation & separation of metabolites



Mass spectrometry (MS)

- High sensitivity (0.5 pg = 1.3×10^{-15} mol input) ✓
- High specificity (< 5ppm error) ✓

Creatine
$C_4H_9N_3O_2$
m/z = 132.0768



Leucine
$C_6H_{13}NO_2$
m/z = 132.1019

LC/MS is the single most versatile metabolite profiling technique

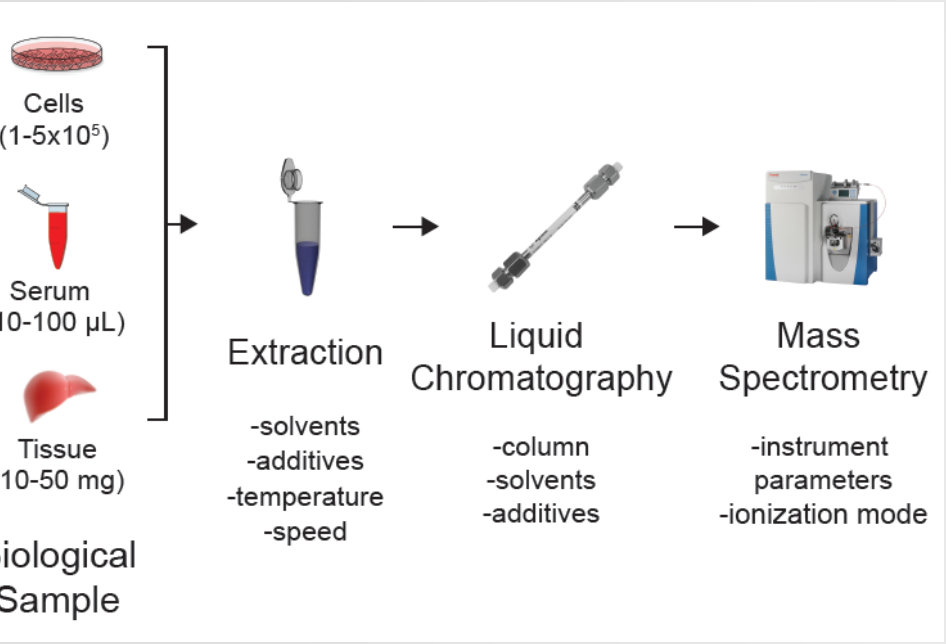
Liquid chromatography (LC) separates isomers

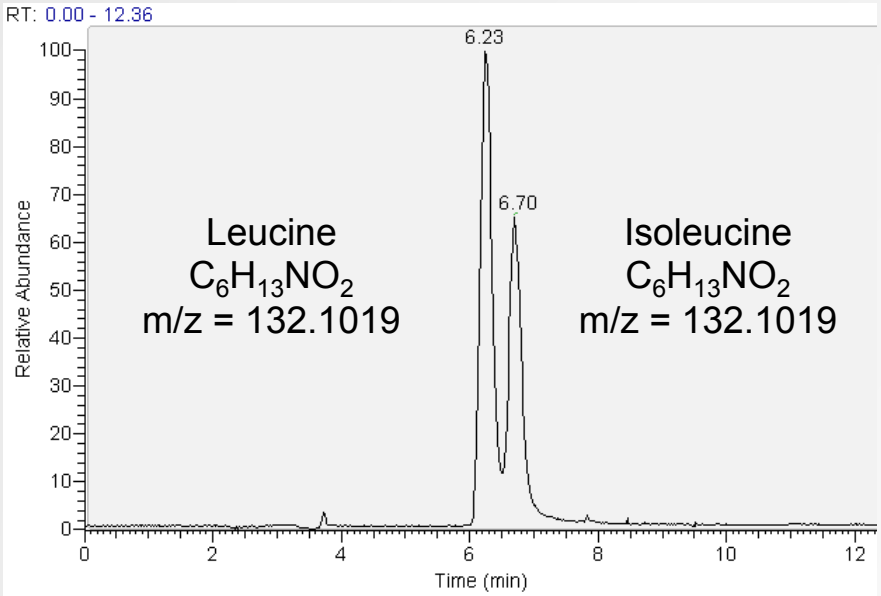
- Ability to choose appropriate conditions for chemically diverse molecules

Mass spectrometry (MS) specifically identifies one or a few metabolites

- Exact mass and/or fragmentation pattern

Peaks can be integrated to quantitatively measure metabolite levels





Metabolomics in precision biomedicine

Metabolic processes as targeted ways to attack disease

Metabolite profiling as a strategy to personalize medicine

Metabolomics in precision biomedicine

Metabolic processes as targeted ways to attack disease

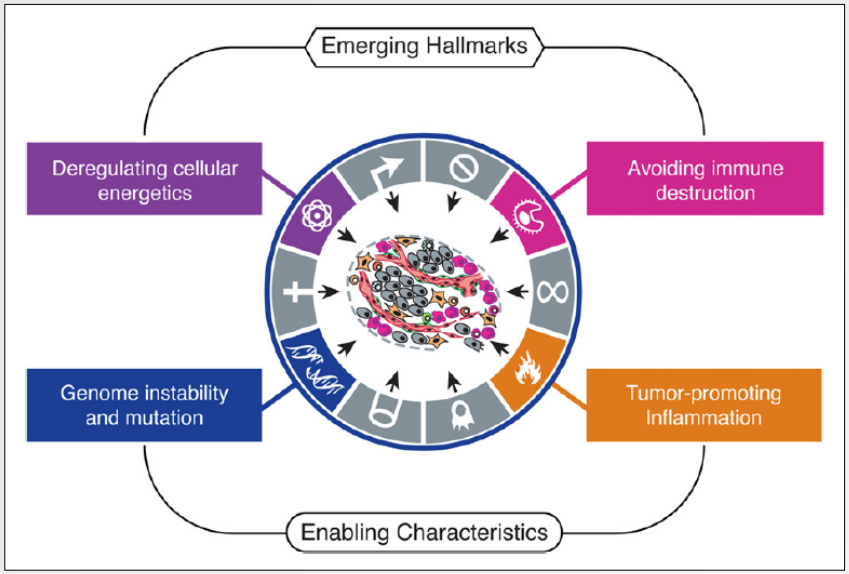
Metabolite profiling as a strategy to personalize medicine

Altered cellular metabolism is an emerging cancer hallmark

Provide building blocks for cell growth & division

Maintain energetic & redox balance

Modulate transcriptional & epigenetic signals to promote survival & growth



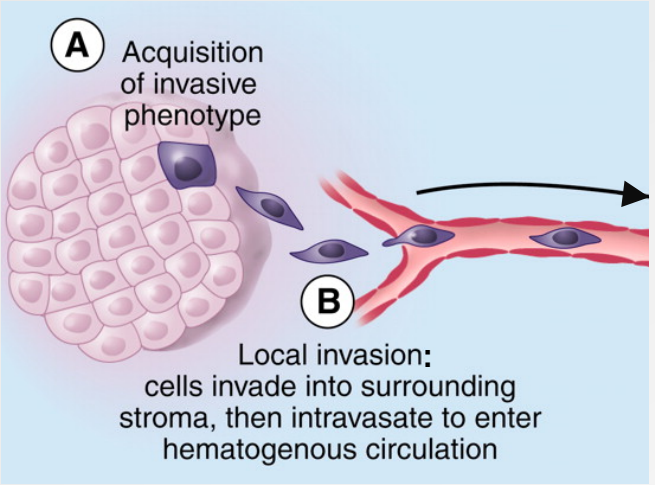
Hanahan and Weinberg, 2011

Do metabolic changes also enable cancer aggressiveness?

- Resistance to chemotherapy & other stressors
- Ability to migrate & seed distant tumors → metastasis

Cancer cells can acquire aggressive traits through the epithelial-mesenchymal transition (EMT)

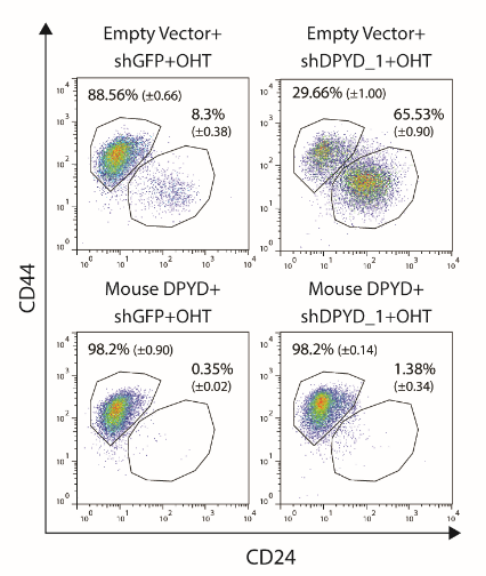
- Normal developmental process re-activated aberrantly in cancer cells
- Loss of polarity & adhesion
- Migration & extravasation
- Resistance to apoptosis
- Ability to seed distant tumors

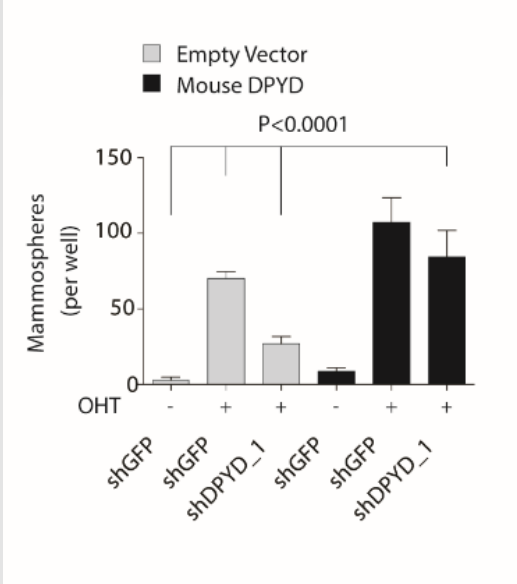


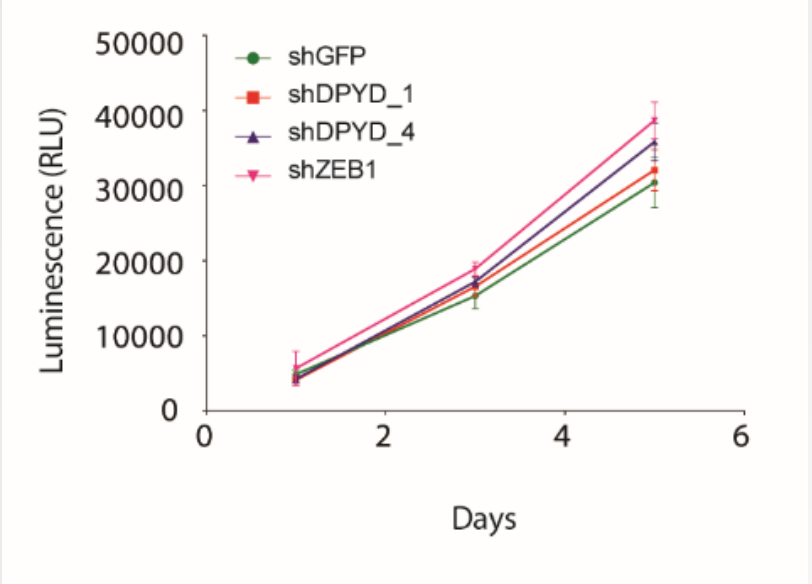
Chaffer and Weinberg, 2011

DPYD is a metabolic gene required for EMT

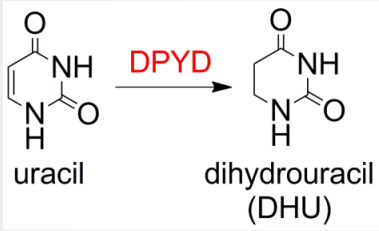
- Identified by gene expression analysis of epithelial- and mesenchymal-like cells
- Used model system in which cultured cells can be induced to undergo EMT
- Knockdown of DPYD inhibits EMT marker expression and mammosphere formation
- Cell proliferation is unaffected







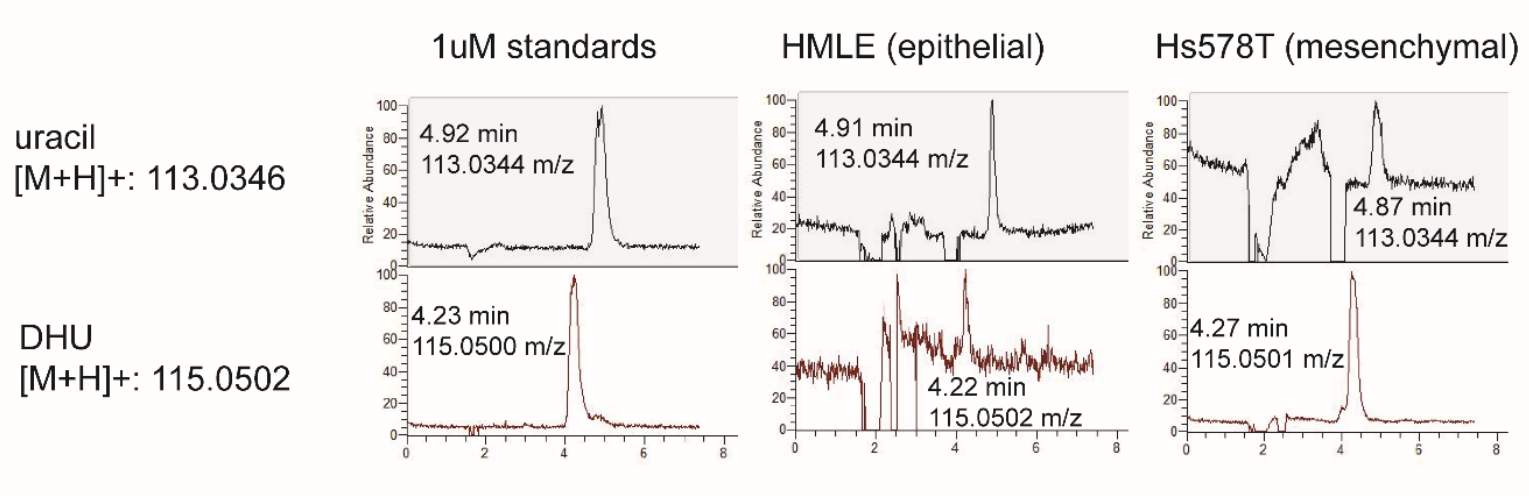
➢ Is DPYD enzymatically active?
Is its activity important for EMT?

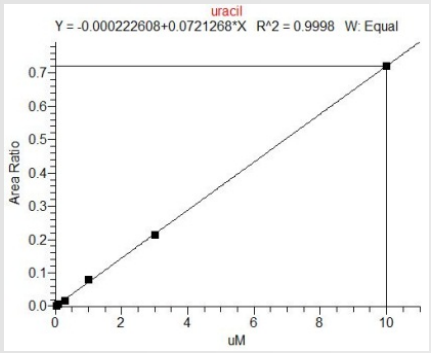


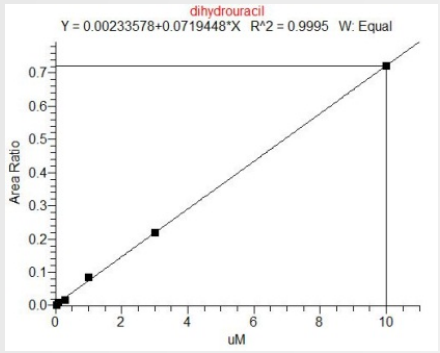
An LC/MS-based assay for DPYD activity in cells

Analysis was challenging because uracil & dihydrouracil abundance is low

Excessive cellular material degrades instrument performance over time





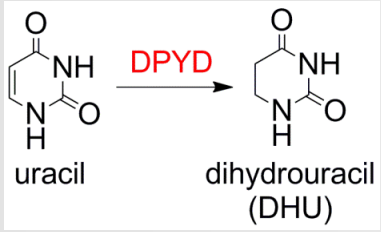


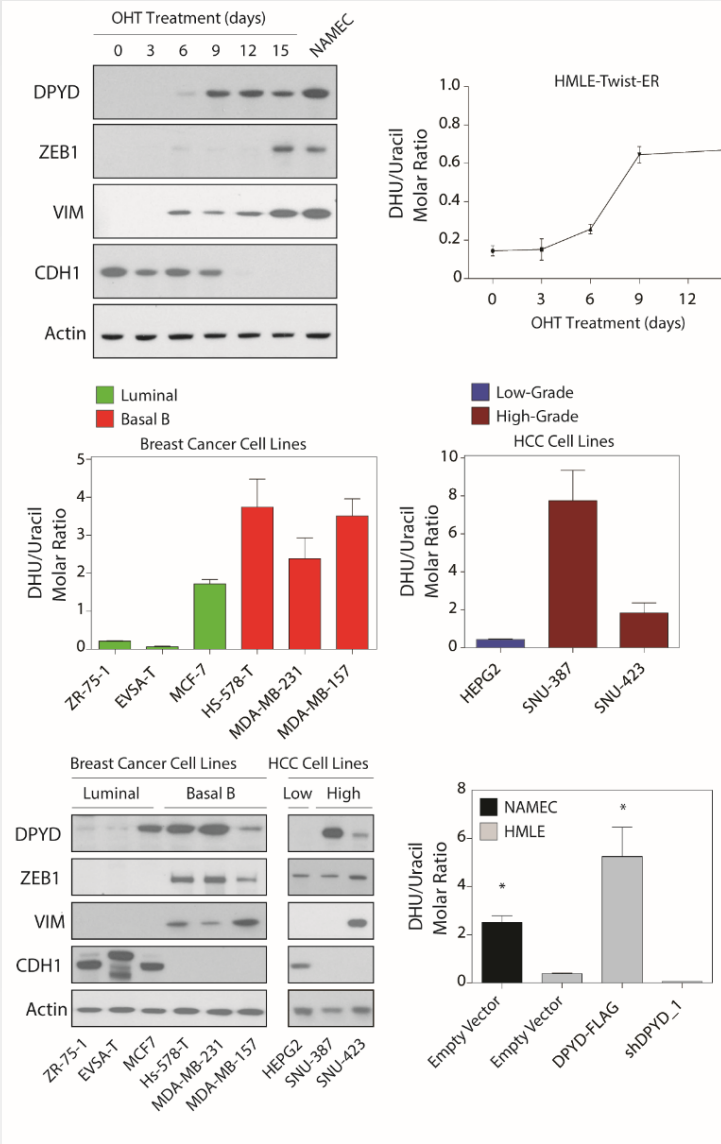
DPYD activity increases during EMT

PYD expression and cellular DHU:uracil atios increase during EMT

lesenchymal-like cell lines express more PYD and have higher DHU:uracil ratios than pithelial-like counterparts

xperimentally manipulating DPYD xpression causes commensurate changes in HU:uracil ratios

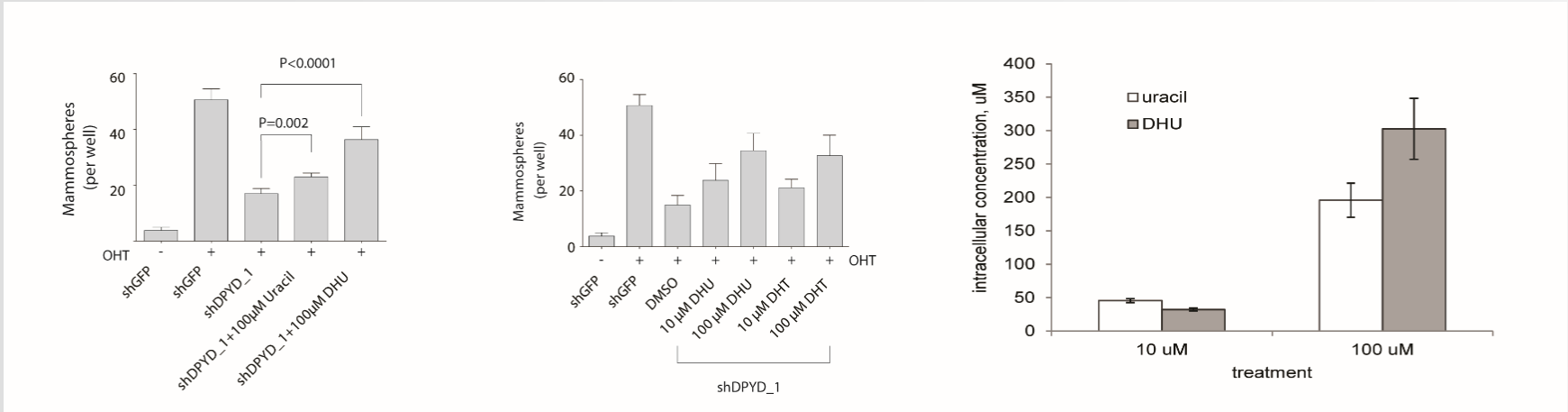




DPYD products specifically promote EMT

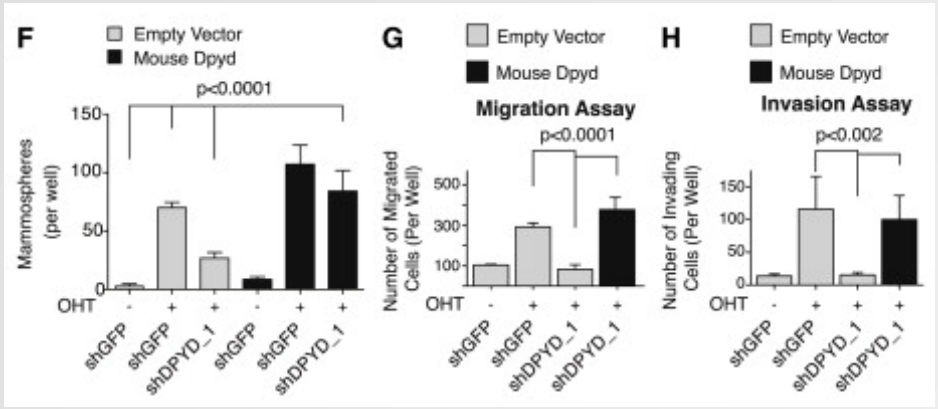
- Dihydropyrimidines added to culture media rescue EMT in DPYD-knockdown cells more potently than pyrimidines

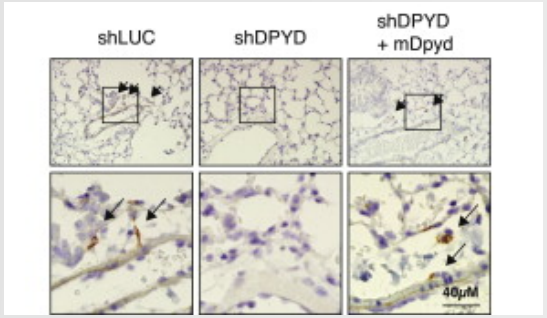
- Both metabolite types accumulate readily inside cells

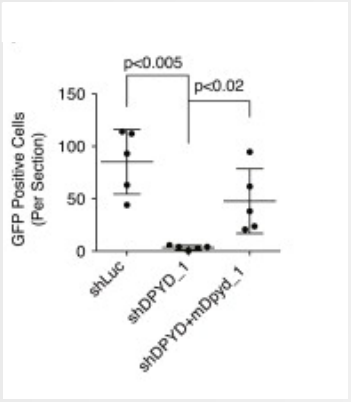


DPYD promotes invasion & metastatic spread of cancer cells in animals

- Clonogenicity, migration, invasion of cultured cells
- Ability to seed lung metastases after tail vein injection in mice







DPYD: conclusions & future directions

- DPYD enzymatic activity and its products are required for carcinoma cells to undergo EMT *in vitro* and *in vivo*
  - First metabolic process required specifically for aggressiveness
- Can pharmacologic inhibition of DPYD prevent or limit metastatic progression?
  - If so, this would not impose a selective pressure on cancer cells to evolve resistance
- Can DPYD expression or product levels serve as a predictive marker for metastasis risk?
- How do DPYD products promote EMT?
- Can other metabolic alterations play a similar role?

Shaul, Freinkman, et al., *Cell* 2014

Metabolomics in precision biomedicine

Metabolic processes as targeted ways to attack disease

Metabolite profiling as a strategy to personalize medicine

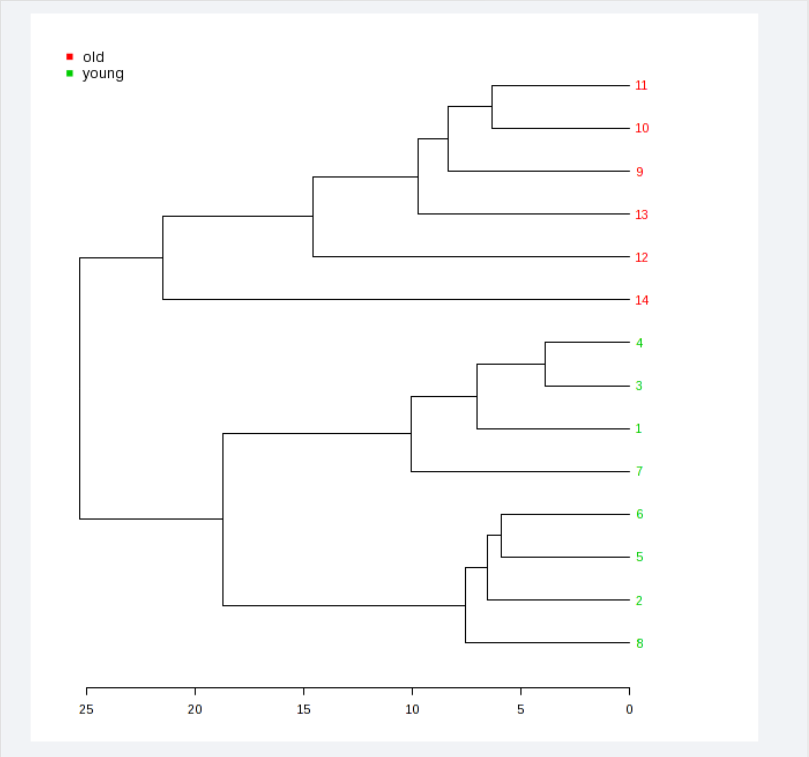
- ➢ Finding differences among patients that are important for therapy
- Disease diagnosis
- Disease subtype
- Disease risk

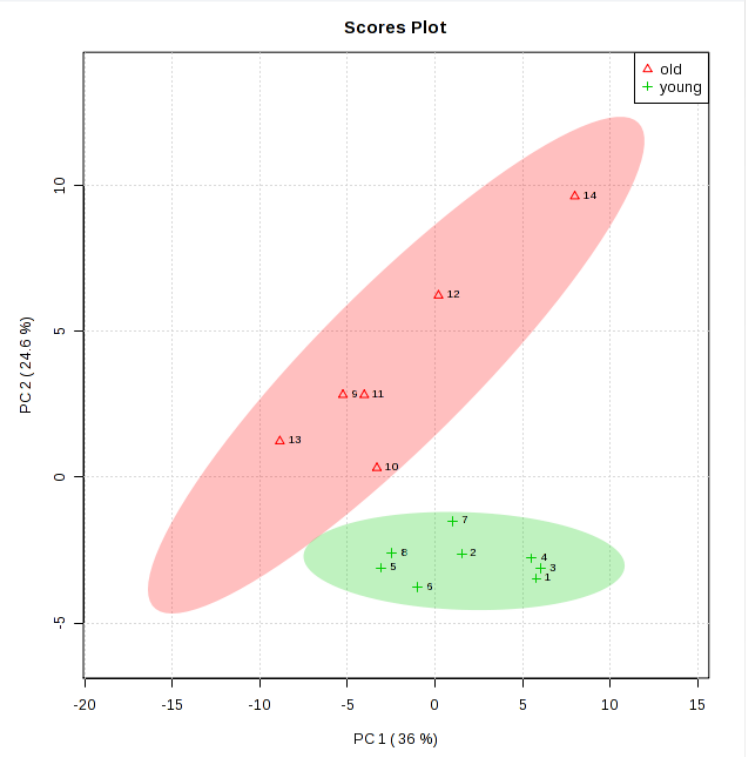
Measure many metabolites and find associations to outcome of interest

- Causal link is often challenging to prove and understand
- Need many measurements and careful controls for statistical significance

ase study: Metabolic signature of aging in mammal

- Collaboration with Dr. Monther Abu Remaileh (Sabatini lab)
- Compared blood plasma from aged vs. young mice
- Measured ~150 known polar metabolites
- Young and old mice display distinct metabolomic signatures





Metabolomics in precision medicine: diagnostics

lividuals with elevated BCAAs
ve a 2-fold increased risk of
ncreatic cancer diagnosis in
owing 2-5 years

uld this finding lead to an “early
rning” diagnostic tool?

- Pancreatic cancer is usually diagnosed too late to treat effectively

LET

nature medicine 2014, 20(10):1193-8.

Elevation of circulating branched-chain amino acids is an e
event in human pancreatic adenocarcinoma development

Jared R Mayers[1,2,23], Chen Wu[3–5,23], Clary B Clish[6,23], Peter Kraft[5,7], Margaret E Torrence[1,2], Brian P Fiske[1,2], Chen Yuan[4], Ying Bao[8], Mary K Townsend[8], Shelley S Tworoger[5,8], Shawn M Davidson[1,2], Thales Papagiannakopoulos[1,2], Annan Yang[9], Talya L Dayton[1,2], Shuji Ogino[4,5,10], Meir J Stampfer[5,8,11], Edward L Giovannucci[5,8,11], Zhi Rong Qian[4], Douglas A Rubinson[4], Jing Ma[5,8], Howard D Sesso[5,12], John M Gaziano[12,13], Barbara B Cochrane[14], Simin Liu[15,16], Jean Wactawski-Wende[17], JoAnn E Manson[5,8], Michael N Pollak[18,19], Alec C Kimmelman[9], Amanda Souza[6], Kerry Pierce[6], Thomas J Wang[20], Robert E Gerszten[6,21], Charles S Fuchs[4,8], Matthew G Vander Heiden[1,2,4,6] & Brian M Wolpin[4,22]

For lay-audience synopsis, see:
http://scitechdaily.com/biologist-reveal-boost-in-certain-amino-acids-is-an-early-sign-of-cancer/

Metabolomics in precision medicine: drug safety

- Identification of drug metabolites for safety testing (pharmacodynamics)

Required for FDA approval

- Specific urine metabolites as early predictors of drug-induced liver injury

J.H. Winnike et al., *Clinical Pharmacology & Therapeutics*, 2010

- Microbiome-specific differences that affect drug metabolism

J.A. Clayton et al., *Proceedings of the National Academy of Sciences*, 2009

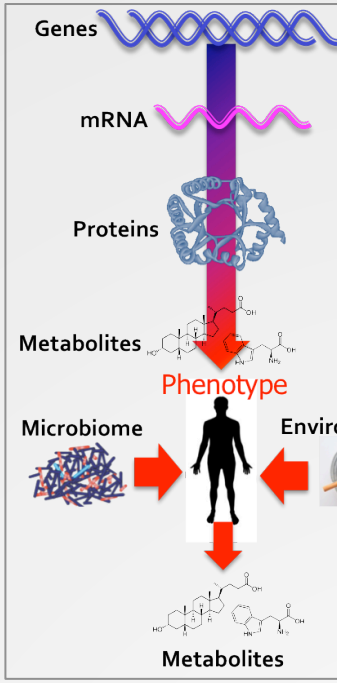
Summary

Metabolomics is a key, emerging “omics” technology

- Complementary to genomics, transcriptomics and proteomics

Metabolomics is contributing important insights throughout the biomedical research process

- Basic research on disease mechanisms & signatures
- Disease risk and diagnosis
- Drug safety and efficacy



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Monther Abu Remaileh

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freinkm@wi.mit.edu
metabolomics.wi.mit.edu