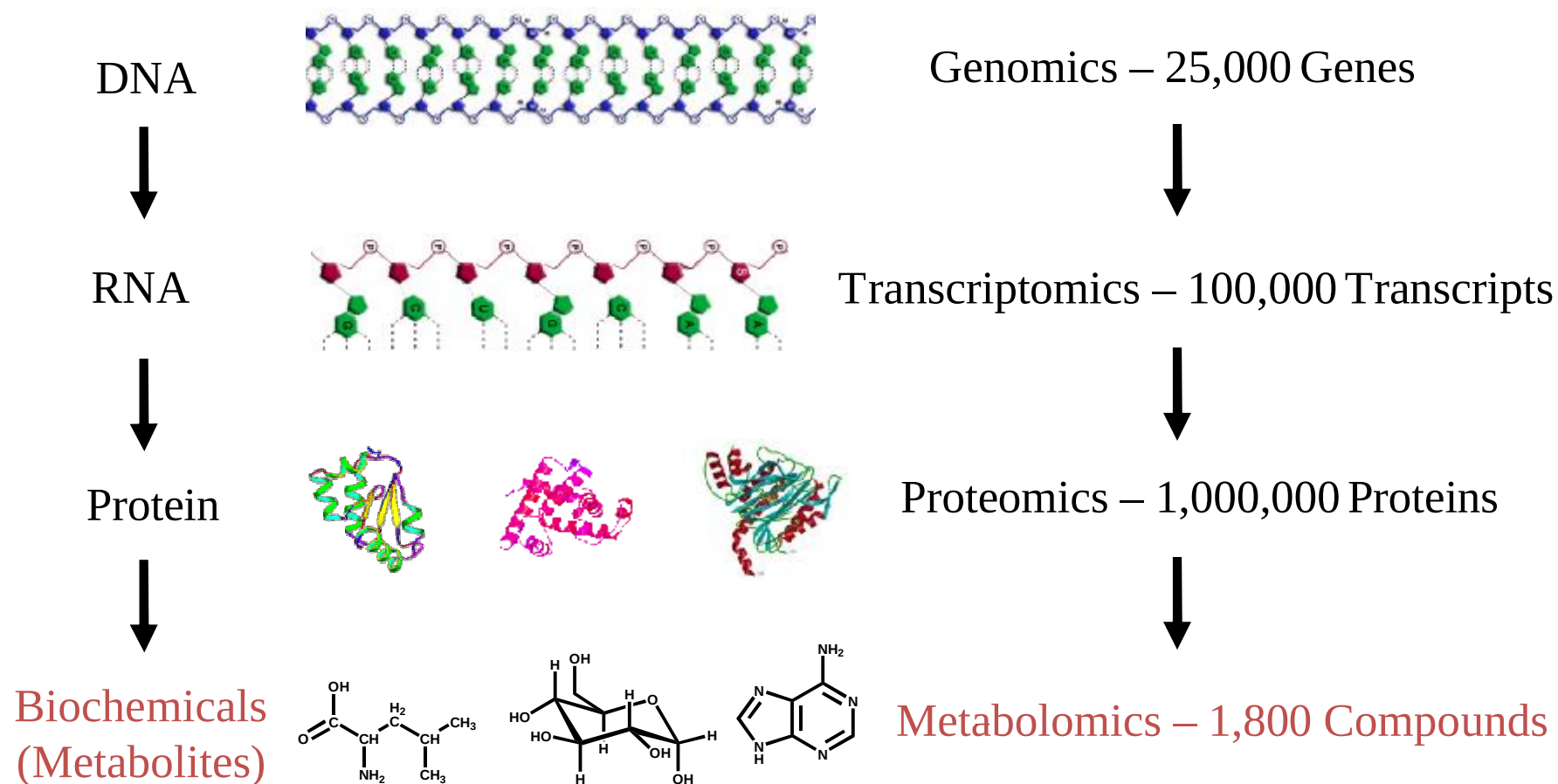


Technological Achievements through Collaboration

Institute of Medicine

July 22, 2010

Metabolomics



Unbiased Metabolomics

- Seeks to identify all chemical entities on a biological sample and comment on their relevance.
 - All chemical entities are characterized irrespective of their identity, and indeed many are unknown.
 - Statistical relevance does not require chemical identity
 - Biochemical relevance does require chemical identity

Experimental design

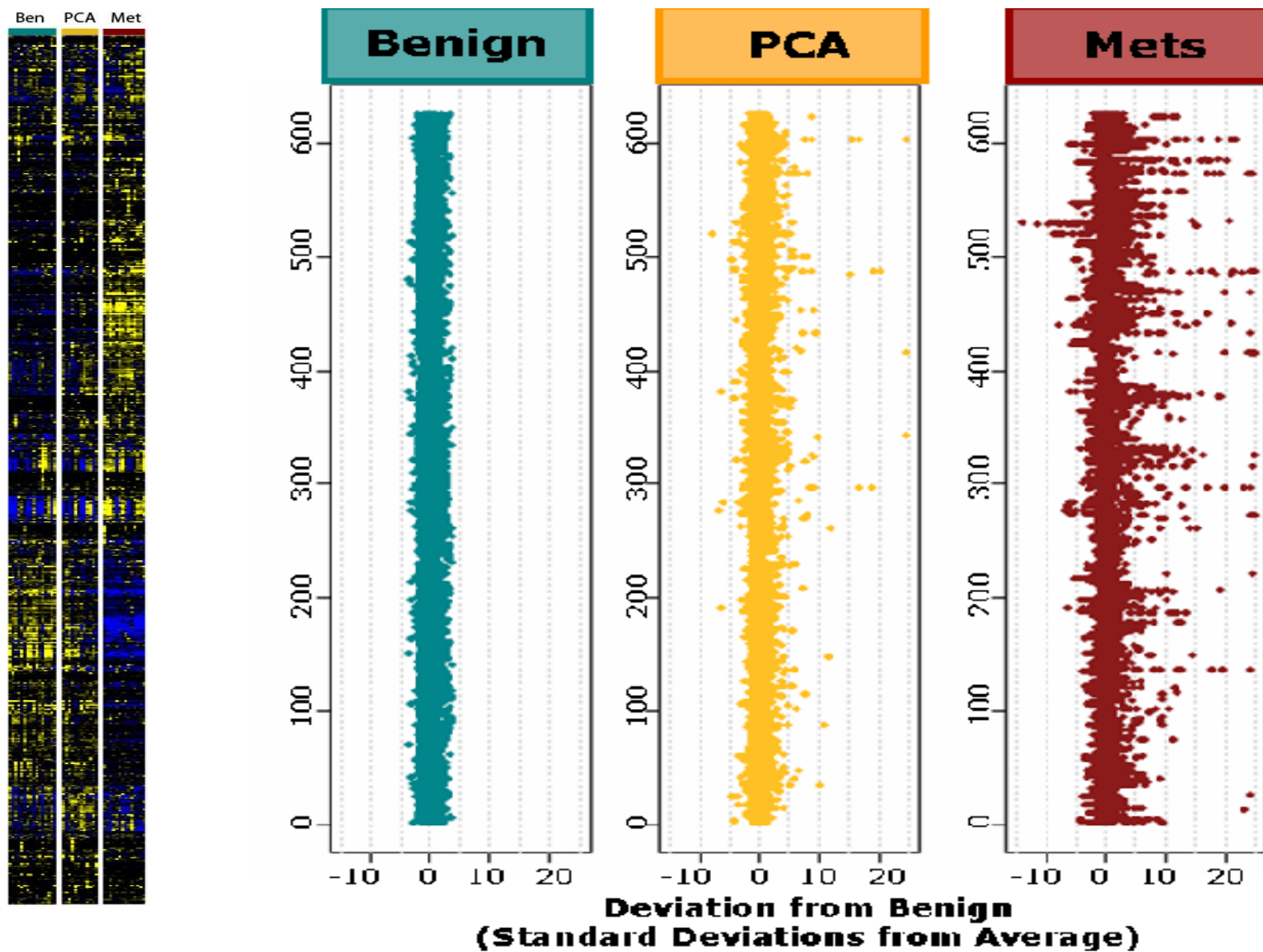
	Prostate Tissues (n=20/class) Benign Low Grade PCA High Grade PCA	Plasma (n=50/class) Biopsy Negative Controls Low Grade PCA High Grade PCA	Post-DRE Urine (n=50/class) Biopsy Negative Controls Low Grade PCA High Grade PCA
Genomics	Transcriptomic/cDNA arrays SNP Chips microRNAs ETS gene fusion analysis (QRT-PCR, FISH) Differential Methylation Hybridization	Will not be done for this biospecimen	ETS gene fusion analysis (QRT-PCR, FISH) QRT-PCR of other candidates
Proteomics	High-throughput immunoblot analysis 2-D liquid phase fractionation/mass spec	Autoantibody Signatures ELISAs	Immunoblot Analysis ELISAs
Metabolomics	Full Platform	Full Platform	Full Platform

Results

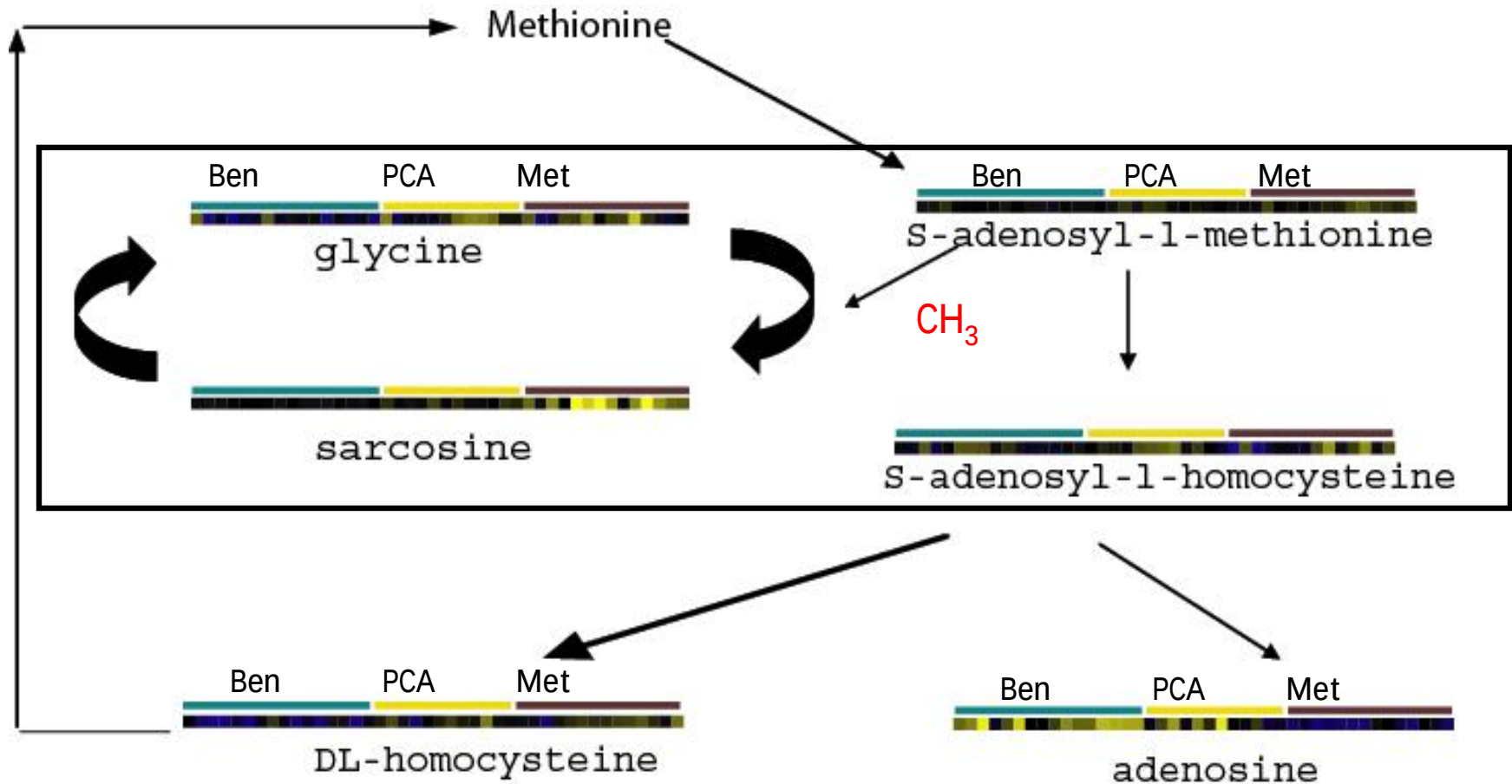
- Using a combination of high throughput liquid and gas chromatography-based mass spectrometry, we profiled more than 1265 metabolites across 262 clinical samples related to prostate cancer (tissue, urine, and plasma).

*Funded by the Early Detection Research Network (EDRN) /NCI

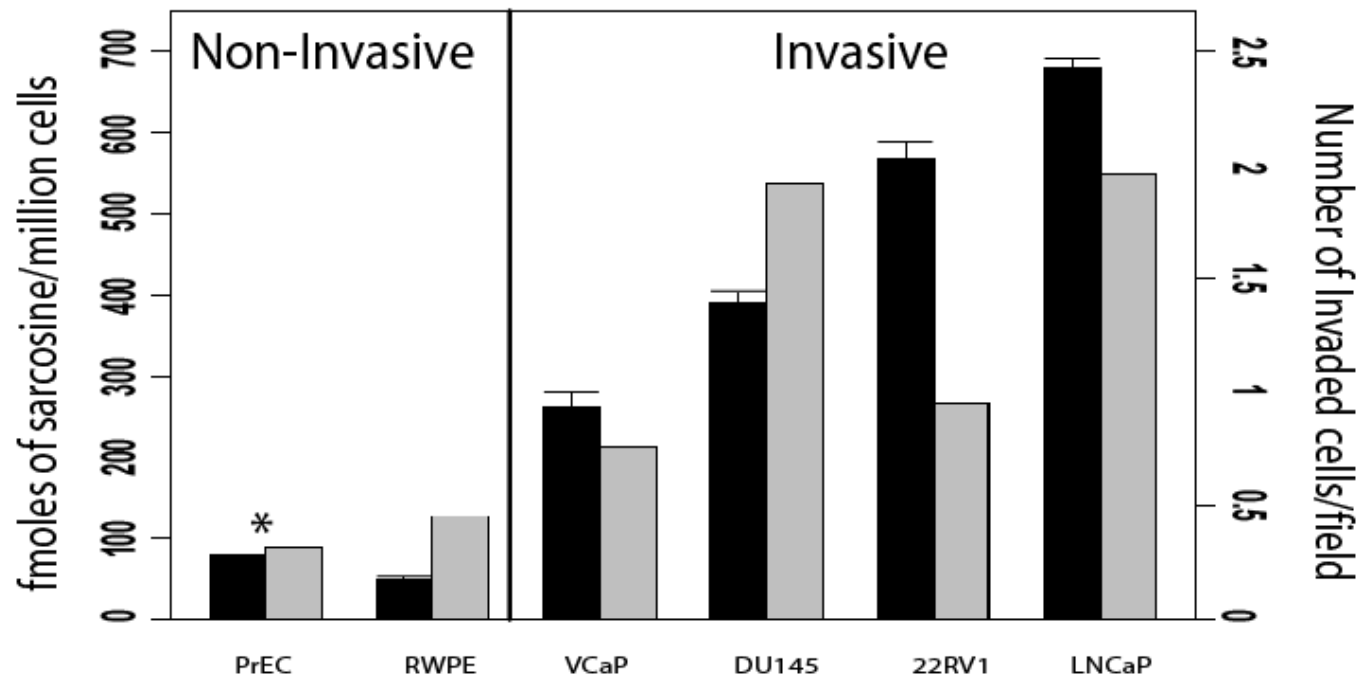
Statistical Differences



Sarcosine: A Methylated derivative of Glycine is elevated in Metastatic Prostate cancer



Elevated Sarcosine Production in Prostate Cancer Cell Lines



■ Sarcosine levels
■ Invasion

EZH2 induces Invasion

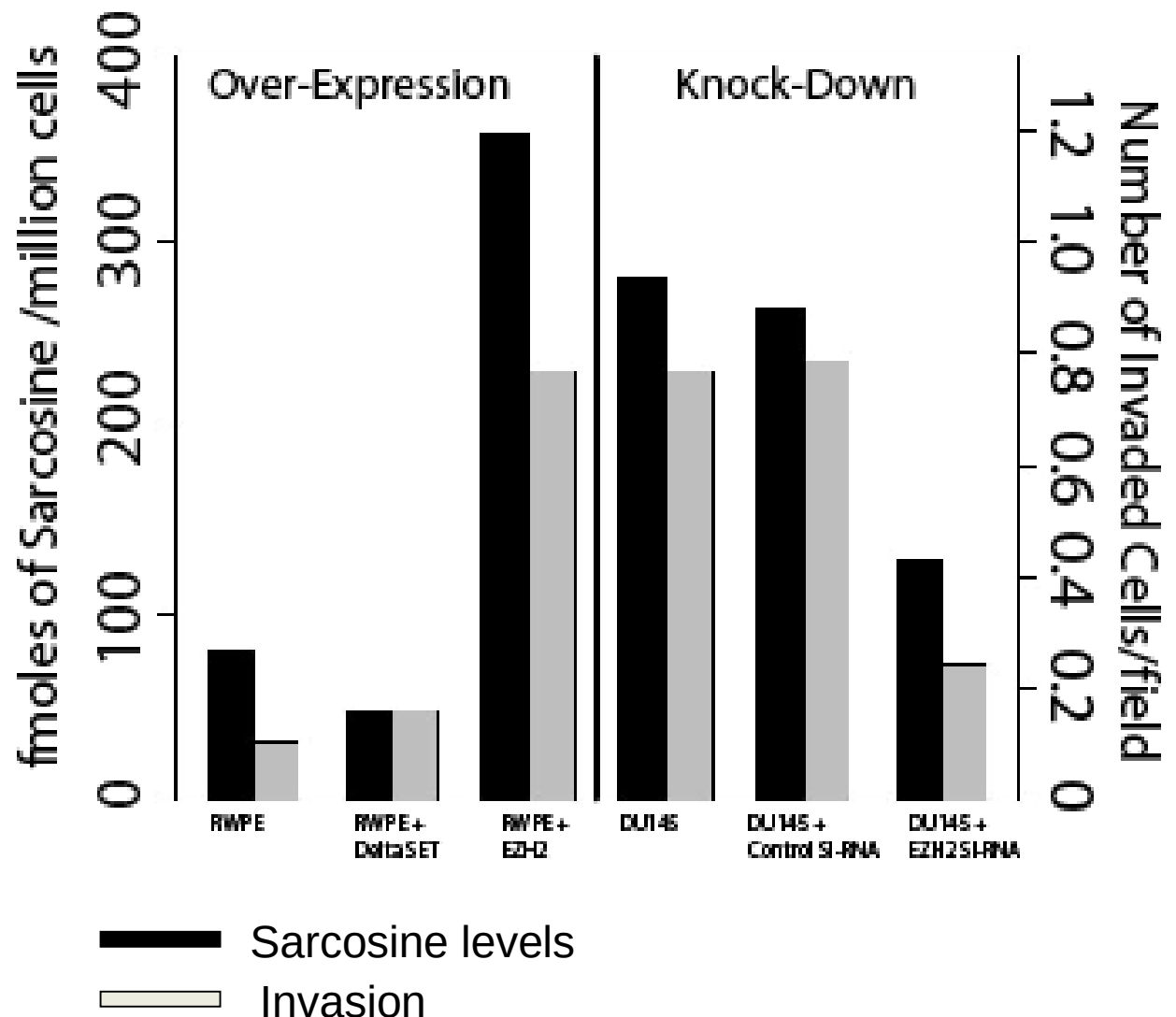
The polycomb group protein EZH2 is involved in progression of prostate cancer

Sooryanarayana Varambally^{†‡}, Saravana M. Dhanasekaran^{†‡}, Ming Zhou^{*}, Terrence R. Barrette^{*}, Chandan Kumar-Sinha^{*}, Martin G. Sanda^{‡§}, Debashis Ghosh^{||}, Kenneth J. Pienta^{‡§¶}, Richard G. A. B. Sewalt[‡], Arie P. Otto[‡], Mark A. Rubin^{†‡§} & Arul M. Chinnaiyan^{†‡§}

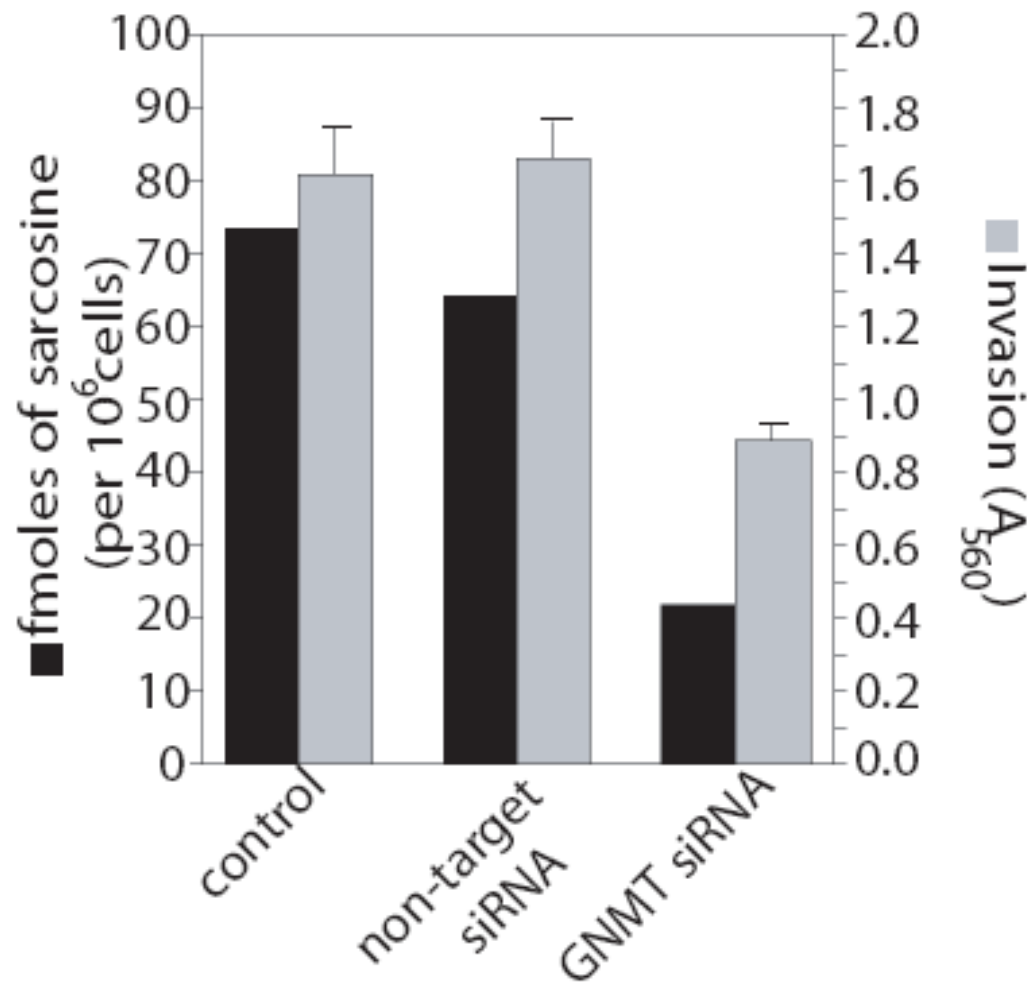
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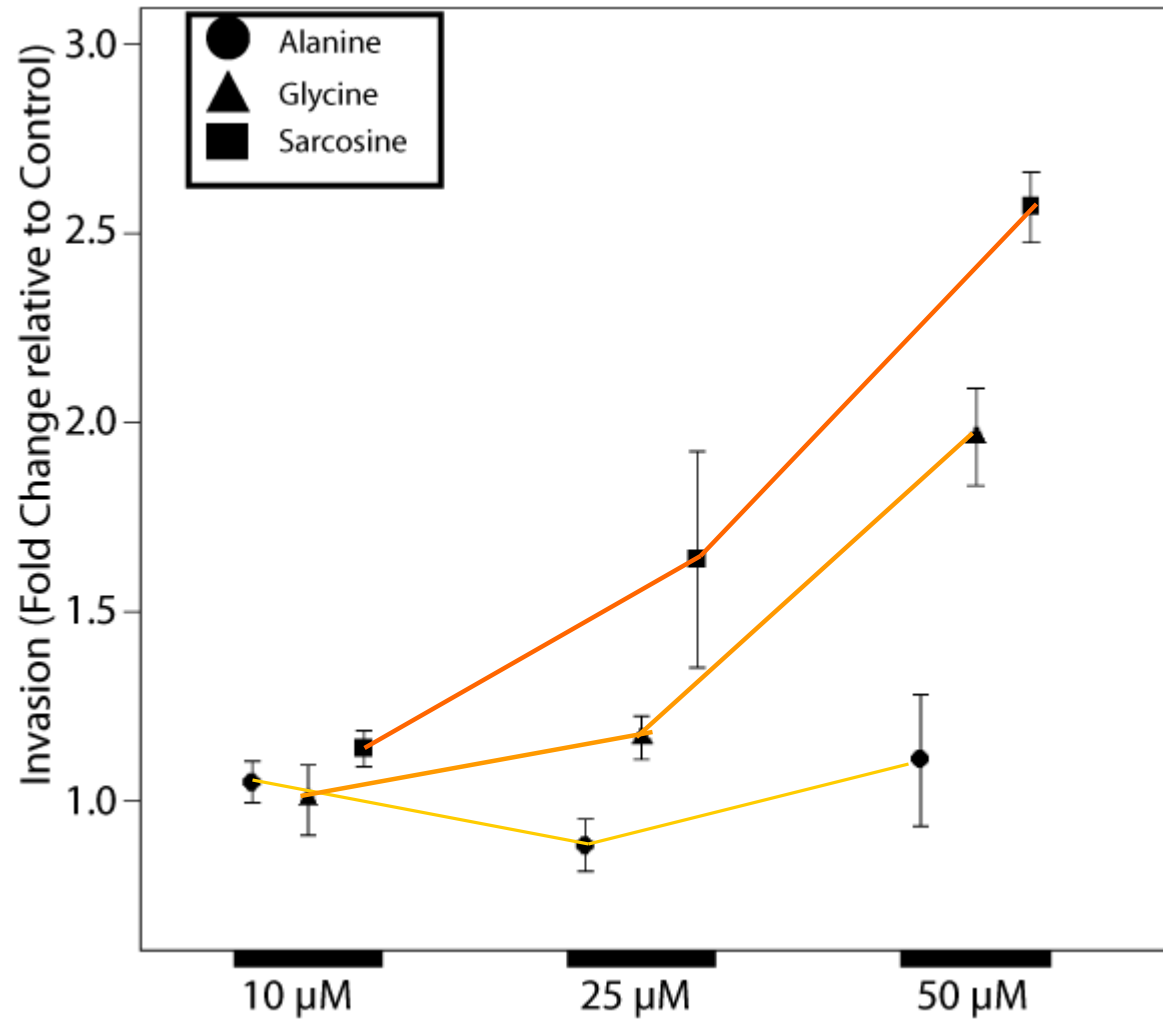
[†] These authors contributed equally to this work



Inhibition of GNMT



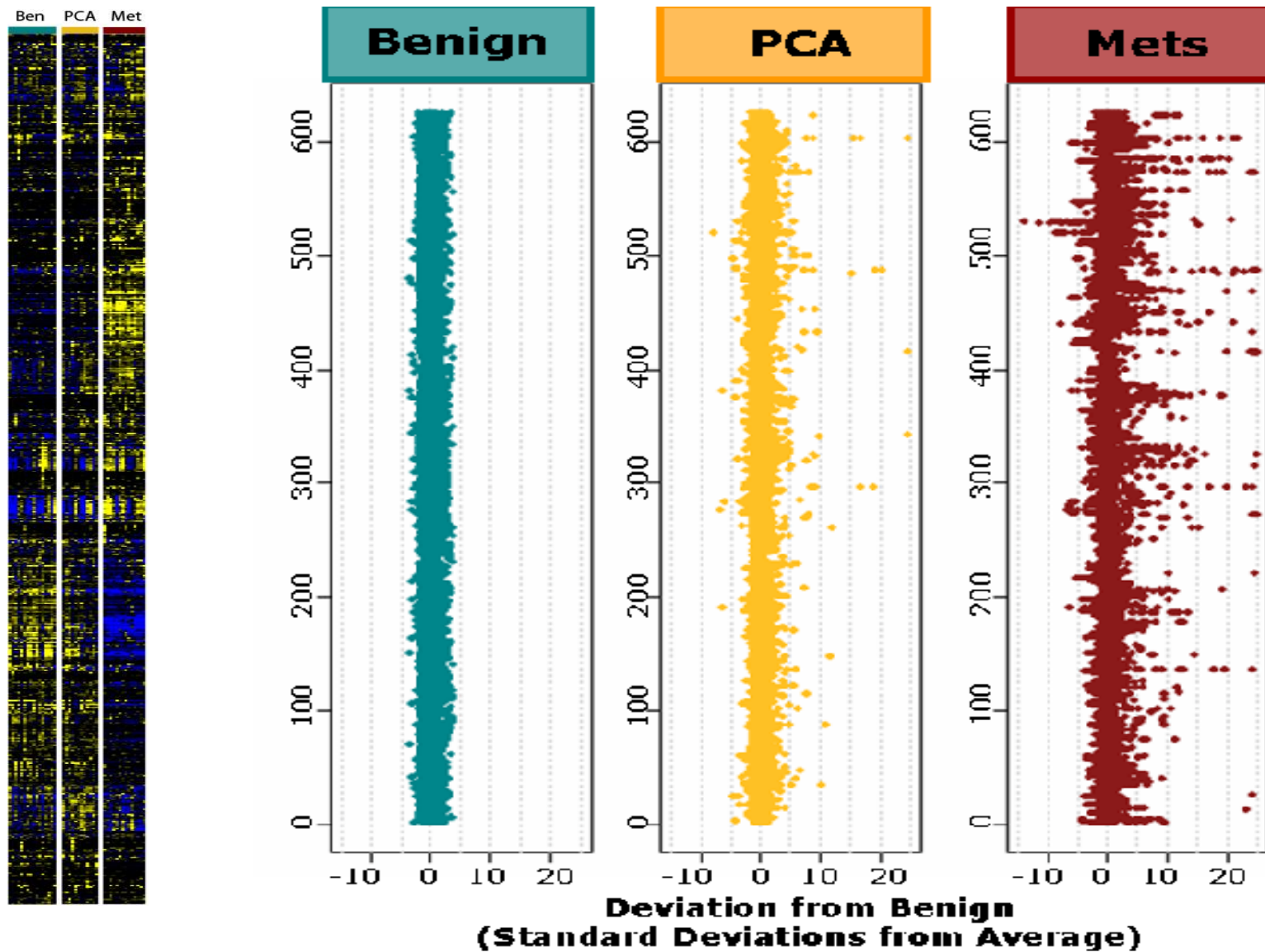
Addition of Sarcosine to Benign Cells



Prostate Conclusions

- Differential metabolomic alterations occur during prostate cancer progression
- Metabolites involved in methylation/epigenetic processes are enriched in prostate cancer progression
- Sarcosine is a key metabolite induced in prostate cancer progression and associated with cancer cell invasiveness
- EZH2 induced cell invasion is associated with elevation of sarcosine
- Sarcosine may be an important metabolic intermediary of cancer cell invasion
- Sarcosine may represent a metabolomic biomarker of aggressive forms of cancer

Statistical Differences





Human Blood Plasma Metabolome (HuPMet) Consortium

Under the direction of:

Professor Christopher Beecher
University of Michigan
Center for Translational Pathology

Convening Partners:

Bristol-Myers Squibb, Pfizer, Takeda Pharmaceuticals,
Human Metabolome Technologies, &
Agilent Technologies

An academic collaboration to:

- 1) Identify and quantitate all of the small molecule components of human blood plasma that are generally found in greater than 0.01 nanomolar concentrations (~2000 compounds)
- 2) Develop reproducible systems/comprehensive platforms/protocols for optimal separation and detection of these molecules
- 3) Determine the “normal” distribution of each molecule, including mean concentration, standard deviation, and modality of distribution

In order to Establish the Consortium:



- Focused on “normal” to reduce IP issues
- Set platform and methodology standards
- Publish final results after embargo period

Thank You

