Metabolism, Metabolomics and Cancer

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Cancer

- A disease caused by an uncontrolled division of abnormal cells in a part of the body
- "The Emperor of All Maladies"
- 41% of us will develop cancer at some point in our lives
- 2\textsuperscript{nd} leading cause of death in US
- Leading cause of death in Canada, UK, New Zealand, Australia, Denmark
44 Years Ago

- Nixon declared war on cancer on Dec 23, 1971
- Since then >$200 billion has been spent on cancer research
39 Years Ago

The Discovery of Oncogenes (Varmus & Bishop ~1976)
Cancer as a Genetic Disease

Oncogenes

Normal cell
- Normal genes regulate cell growth

Cancer cell
- Oncogenes accelerate cell growth and division
- Mutated/damaged oncogene

National Cancer Institute
Cancer as a Genetic Disease

- Every cancer cell has mutations leading to overexpression or perturbations to oncogenes, proto-oncogenes or tumor suppressor genes
- An oncogene is a gene that has the potential to cause cancer
- A proto-oncogene is a normal gene that can become an oncogene due to mutations or increased expression
- A tumor suppressor gene (TSG) is a normal gene that prevents tumor development
- Examples of oncogenes include: Ras, Myc, Raf, Src, EGFR, HER2/neu, HIF-1α, Wnt, Erk, Trk, Bcr-Abl
- Examples of TSGs include: BRCA1, p53, PTEN
June 26, 2000 – 1st Draft of Human Genome Completed
15 Years Ago

The Hallmarks Of Cancer

Sustaining proliferative signaling
Evading growth suppressors
Resisting cell death
Activating invasion and metastasis
Inducing angiogenesis
Enabling replicative immortality

Unbounded Optimism

Time Magazine
April 1, 2003
New Cancer Therapies

- Gene therapy
- T-cell therapy
- Stem cell transplant
- Monoclonal antibody therapy
  - Rituximab
  - Campath
- Mitotic inhibitors
  - Paclitaxel
  - Vinblastine
- Topoisomerase inhibitors
  - Irinotecan
  - Etopiside
- Anti-hormone therapy
  - Tamoxifen
- Targeted wonder drugs
  - Gleevec
5 Years Ago

Next Generation DNA Sequencing

ABI SOLiD - 20 billion bases/run
Sequencing by ligation

Illumina/Solexa 15 billion bases/run
Sequencing by dye termination
The Cancer Genome Atlas
The Good News

![Cancer Survival Rates Graph](source-macmillan-cancer-support)
However…

• Most improvements in cancer survival are due to better screening, which leads to earlier detection (stage I or II), which leads to statistically longer survival times

• Most advances in “curing” cancer have been seen in relatively rare cancers (childhood leukemia, certain types of lymphomas)
Not So Good News

Cancer Survival Rates

- All cancers
- Adult leukaemia
- Ovary
- Myeloma
- Lung
- Oesophagus
- Others
- Stomach
- Pancreas
- Brain

Source: Macmillan Cancer Support

Years:
- 1971-72
- 1980-81
- 1990-91
- 2000-01
- 2007
The Bad News

Age-Adjusted Death Rates (US)
The Really Bad News

- Cancers are caused by 2-3 “founder” mutations (to oncogenes/TSPs)
- ~250 oncogenes, ~700 tumor suppressor genes identified so far
- Cancer is 1,000,000+ different diseases
- Cancer cells accumulate ~10,000-50,000 mutations/CNVs after conversion (genetic noise)
- Cancer cells are a “genetic train wreck”
Where To Next?

Genomics

Less Traveled
How Was Cancer Viewed Prior to 1970?

• Prevailing opinion among most oncologists was that cancer was a “metabolic disease”

• Cancer cells were metabolically dysregulated (cause of the metabolic dysregulation was unknown)

• Cancer drugs were called “anti-metabolites” and cancer chemotherapy was called anti-metabolite therapy
# Anti-Metabolite Cancer Drugs

<table>
<thead>
<tr>
<th>Anti-metabolite</th>
<th>Metabolite equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Fluorouracil (5-FU) - 1957</td>
<td>Uracil</td>
</tr>
<tr>
<td>Gemcitabine (Ara-C) - 1981</td>
<td>Cytosine</td>
</tr>
<tr>
<td>6-Mercaptopurine - 1951</td>
<td>Adenine/Guanine</td>
</tr>
<tr>
<td>Fludarapine (Ara-A) - 1968</td>
<td>Adenine</td>
</tr>
<tr>
<td>Methotrexate - 1956</td>
<td>Folate</td>
</tr>
<tr>
<td>Aminopterin - 1947</td>
<td>Folate</td>
</tr>
<tr>
<td>Megestrol acetate - 1956</td>
<td>Progesterone</td>
</tr>
<tr>
<td>Asparaginase* - 1963</td>
<td>Asparagine/Glutamine*</td>
</tr>
</tbody>
</table>
Who Came Up With This Crazy Idea?

• Observed in 1924 that cancer cells use aerobic glycolysis to fuel growth instead of oxidative phosphorylation

• Won the Nobel Prize in 1931

• Advocated that: “replacement of oxygen-respiration by fermentation is the prime cause of cancer”

• The metabolic view of cancer predominated thinking from 1920’s up to Warburg’s death in 1970

Otto Warburg
Cancer is a Metabolic Disease

- Cancer cells consume 100-200X more glucose than other cells in the body.
- This unique metabolism is the basis to PET (positron emission tomography) scans for cancer using fluorinated deoxyglucose.
- This metabolic shift is called the Warburg effect or cytosolic aerobic glycolysis.

Tumors are marked in black in this PET image (lots of glucose).
How Is A Metabolic View of Cancer Compatible With the Genetic View?
## Oncogenes are Metabolic Hubs

<table>
<thead>
<tr>
<th>Oncogene or Tumor Suppressor</th>
<th>Metabolic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt</td>
<td>Enhances glucose uptake, activates hexokinase II</td>
</tr>
<tr>
<td>c-Myc</td>
<td>Enhances glycolysis, activates LDH-A</td>
</tr>
<tr>
<td>h-Ras, k-Ras</td>
<td>Enhances glycolysis, activates complex II</td>
</tr>
<tr>
<td>Src</td>
<td>Phosphorylates PKM2, upregulates c-Myc</td>
</tr>
<tr>
<td>Brc-abl</td>
<td>Enhances glucose uptake, activates G6PD &amp; HK II</td>
</tr>
<tr>
<td>Her2/neu</td>
<td>Enhances glycolysis, activates LDH and HSF1</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>Sustains TCA cycle, loss leads to HIF activation</td>
</tr>
<tr>
<td>Fumarate hydratase</td>
<td>Sustains TCA cycle, loss leads to HIF activation</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>Sustains TCA cycle, loss leads to DNA methylation</td>
</tr>
<tr>
<td>p53</td>
<td>Promotes OXPHOS, loss leads to glycolysis</td>
</tr>
</tbody>
</table>
Updated Hallmarks of Cancer

Normal Cell Metabolism

Glucose

Glucose-6-P

Fructose-6-P

Fructose-1,6-BP

Glyceraldehyde-3-P

1,3-Diphosphoglycerate

3-Phosphoglycerate

Phosphoenolpyruvate

Pyruvate

Hexokinase-2

Isomerase

Phosphofructokinase

Aldolase

GAPDH

Phosphoglycerate kinase

Enolase

Pyruvate Kinase

HCO₃

pH 7.4

CO₂

Carbonic Anhydrase

ATP

Acetyl-CoA

Citrate

α-ketoglutarate

Oxaloacetate

Malate

PDH

Pyruvate

Pyruvate Kinase
Cancer Cell Metabolism

Glucose → Glucose-6-P → Fructose-6-P → Fructose-1,6-BP → Glyceraldehyde-3-P → 1,3-Diphosphoglycerate → 3-Phosphoglycerate → Phosphoenolpyruvate → Pyruvate

Hexokinase-2 → Isomerase → Phosphofructokinase-1 → Aldolase → GAPDH → Citrate Lyase

Pyruvate Kinase → Pyruvate → Lactate

Pyruvate → Acetyl-CoA → Fatty acids → Oxaloacetate → α-ketoglutarate

Glutamine → Glutamate → Lactate → Pyruvate

Malic Enzyme → Pyruvate → Oxaloacetate → α-ketoglutarate

Glyceraldehyde-3-P → Lactate → Pyruvate → Carbonic Anhydrase

pH 6.5 → MCT4

Slca1

Acetyl-CoA → Fatty acid Synthase → Fatty acids

Glutamine → Glutamate → Glutaminase

Malic Enzyme

Pyruvate Kinase M2
How To Measure All These Metabolic Changes?
Answer: Metabolomics

- Metabolomics
- Proteomics
- Genomics

Influences:
- Environmental
- Physiological
Measuring Metabolism with Metabolomics

Biological or Tissue Samples → Extraction → Biofluids or Extracts

Data Analysis → Chemical Analysis
Human Metabolomes (2015)

- 3670 (T3DB) Endogenous metabolites
- 1240 (DrugBank) Drug metabolites
- 28500 (FooDB) Food additives/Phytochemicals
- 1550 (DrugBank) Drugs
- 29700 (HMDB) Endogenous metabolites

Concentration units: M (Molar), mM (Millimolar), µM (Micromolar), nM (Nanomolar), pM (Picomolar), fM (Femtomolar)
Metabolomics & Cancer

Normal | Cancer

<p>| Tyrosine | Leucine | Threonine | Proline | Tryptophan | Histidine | Valine | Isoleucine | Aspartate | Phenylalanine | Glutamate | Glucose | GTP | Creatinine | NAD+ | Alanine | Taurine | Glutamine | NADP+ | ATP | Creatine | Glycine | Succinate | Malate | Fumarate | Lactate | AMP | Asparagine | ADP |
|---------|---------|-----------|---------|------------|-----------|--------|------------|-----------|---------------|-----------|---------|-----|-----------|------|---------|--------|-----------|-------|----|----------|-------|--------|--------|------|-------------|---|</p>
<table>
<thead>
<tr>
<th>Oncometabolite</th>
<th>Effect or Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>Promotes tumor metastasis</td>
</tr>
<tr>
<td>2-Hydroxyglutarate</td>
<td>Alters histone/DNA methylation</td>
</tr>
<tr>
<td>Fumarate</td>
<td>HIF activation/alters DNA methylation/bind GSH</td>
</tr>
<tr>
<td>Succinate</td>
<td>HIF activation/alters DNA methylation</td>
</tr>
<tr>
<td>Glucose</td>
<td>Fuels Warburg effect</td>
</tr>
<tr>
<td>Sarcosine</td>
<td>Promotes tumor metastasis</td>
</tr>
<tr>
<td>Kynurenine</td>
<td>Activates aryl hydrocarbon receptor, tumorigenesis</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Fuels glutaminolysis, promotes tumor growth</td>
</tr>
<tr>
<td>Glycine/Serine</td>
<td>Promotes tumor growth, reverse Warburg effect</td>
</tr>
</tbody>
</table>
Metabolomics is Discovering Cancer Biomarkers

- Vanillylmandelic acid (neuroblastoma + pheochromocytoma)
- 3-Hydroxymandelic acid (neuroblastoma)
- 3,4-Dihydroxymandelic acid (neuroblastoma)
- Homovanillic acid (neuroblastoma)
- Sarcosine (metastatic prostate cancer)
- 2-hydroxyglutarate (glioma + acute myeloid leukemia)
- Ribothymidine (breast cancer)
- 1-methylguanosine (breast cancer)
- 1-methyladenosine (cholangioma + cervical cancer)
- Cadaverine (pancreatic cancer)
- 5-hydroxyindoleacetic acid (carcinoid tumors)
- 3-methoxytyramine (carcinoid tumors)
- Testosterone glucuronide (adrenocortical tumors)
- 3a,16a-dihydroxyandrostenone (adrenal carcinoma)
- 5-methoxyindoleacetate (lung + stomach + colon cancer)
- 21-deoxycortisol (testicular cancer)
- 3,5-diiodothyronine (brain tumors)
- Androstendione (thyroid cancer)
- Thromboxane A2 (Hepatocellular carcinoma)
- Deoxypyridinoline (Multiple myeloma)
Cancer & Metabolite Biomarkers
Building Better Biomarkers

Abstract
Metabolomics is increasingly being applied towards the identification of biomarkers for disease diagnosis, prognosis and risk prediction. Unfortunately among the many published metabolomic studies focusing on biomarker discovery, there is very little consistency and relatively little rigor in how researchers select, assess or report their candidate biomarkers. In particular, few studies report any measure of sensitivity, specificity, or provide receiver operator characteristic (ROC) curves with associated confidence intervals. Even fewer studies explicitly describe or release the biomarker model used to generate their ROC curves. This is surprising given that for biomarker studies in most other biomedical fields, ROC curve analysis is generally considered the standard method for performance assessment. Because the ultimate goal of biomarker discovery is the translation of those biomarkers to clinical practice, it is clear that the metabolomics community needs to start "speaking the same language" in terms of biomarker analysis and reporting-especially if it wants to see metabolite markers being routinely used in the clinic. In this tutorial, we will first introduce the concept of ROC curves and describe their use in single biomarker analysis for clinical chemistry. This includes the construction of ROC curves, understanding the meaning of area under ROC curves (AUC) and partial AUC, as well as the calculation of confidence intervals. The second part of the tutorial focuses on biomarker analyses within the context of metabolomics. This section describes different statistical and machine learning strategies that can be used to create multi-metabolite biomarker models and explains how these models can be assessed using ROC curves. In the third part of the tutorial we discuss common issues and potential pitfalls associated with different analysis methods and provide readers with a list of nine recommendations for biomarker analysis and reporting. To help readers test, visualize and explore the concepts presented in this tutorial, we also introduce a web-based tool called ROCET (ROC Curve Explorer & Tester, http://www.rocet.ca). ROCET was originally developed as a teaching aid but it can also serve as a training and testing resource to assist metabolomics researchers build biomarker models and conduct a range of common ROC curve analyses for biomarker studies.

KEYWORDS: AUC; Biomarker analysis; Biomarker validation and reporting; Bootstrapping; Confidence intervals; Cross validation; Optimal threshold; ROC curve; Sample size

PMID: 23543913 [PubMed]   PMCID: PMC3608878   Free PMC Article
Assessing Biomarkers with ROC Curves

- Plots sensitivity (%TP) vs. specificity (%TN)
- A poor ROC curve would be a straight line with a slope of 1
- The area under an ROC (AUROC) curve is a good measure of the quality of the biomarker
- AUCs of >0.75 are good, AUCs of 0.5 are terrible, AUCs of 1.00 are perfect
AUCs of Common Tests

Mammogram (Benign vs. Malignant)

AUC = 0.53

PSA Test

AUC = 0.65
How Does Metabolomics Do?
Diagnosing Pancreatic Cancer

- Adult Serum Samples
- 43 cases, 41 controls
- NMR metabolomics
- AUC = 0.84 using 8 metabolites
- Glutamate, acetone, 3-hydroxybutyrate, glucose, glutamine, creatine, phenylalanine, formate

Diagnosing Esophageal Cancer

- Adult Urine Samples
- 44 cases, 75 controls
- NMR metabolomics
- AUC = 0.98 using 7 metabolites
- Urea, acetate, acetone, formate, succinate, pantothenate, 2-hydroxyisobutyrate

Diagnosing Endometrial Cancer

- **Adult Serum Samples**
- **40 cases, 41 controls**
- **MS metabolomics**
- **AUC = 0.88 using 3 metabolites**
  - C18:2, PC ae C40:1, C6 (C4:1-DC)
- **Very strong correlation with BMI**
- **Pap smear AUC=0.55**

Predicting Colon Cancer (Polyps)

- Adult Urine Samples
- 162 cases, 422 controls
- NMR metabolomics
- AUC = 0.75 using 17 metabolites
- Butyrate, serine, methanol, beta-alanine, methylhistidine, 3-hydroxybutyrate, acetone, benzoate

Cancer Cachexia

- Adverse metabolic effect from cancer (negative energy balance due to tumor burden, loss of skeletal muscle mass)
- Responsible for significant morbidity and significantly earlier mortality
- Early detection, prediction & prevention could save lives
Predicting Cancer Cachexia via Metabolomics

- Adult Urine Samples
- All with cancer
- 44 cachetic, 29 non-cachectic
- NMR metabolomics
- AUC = 0.90 using 8 metabolites
- Creatine, creatinine, branched chain AAs, glucose

Using Metabolomics to Phenotype Cancer

- Most cancers generate large quantities of glycolysis biomarkers (lactate, formate, glucose, succinate)
- Some cancers produce large quantities of glutaminolysis biomarkers (glutamate, glutamine)
- Certain cancers exhibit dysregulated one-carbon metabolism biomarkers (choline, sarcosine, glycine, serine, hydroxyglutarate)
- Most cancers produce excesses of metabolites belonging to certain cell classes (indoleacetate, homovanillate)
- MRS (chemical shift) & PET imaging or metabolite profiling allows precise phenotyping of cancers
Using Metabolomics To Phenotype Those At Risk

• Is there a metabolome that predisposes one to cancer?
• How to measure the GxE interactions via metabolomics?
• Metabolites that harm: oncometabolites, uremic toxins, transformed xenobiotics
• Metabolites that heal: butyrate, bicarbonate, uric acid, glutathione
But Metabolomics Tests Will Never Be Approved...
Almost Everyone <25 Has Had A Metabolomic Test

Newborn Screening
Metabolomics is Moving to the Bedside

- Number of “approved” tests arising from Metabolomics/Clinical Chem. – 195
- Number of “approved” tests arising from or using Genomics – 100-110
- Number of “approved” single Protein tests (ELISA) – 60
- Number of “approved” tests arising from or using Transcriptomics – 5
- Number of “approved” tests arising from or using Proteomics - 0
Re-Thinking Precision Medicine

BRCA1/2 Testing

Cancer Phenotyping
Key Points

- **Cancer is a metabolic disease**
  - Cancer cells exhibit a 200x increase in glucose consumption
  - Most known oncogenes and tumor suppressors fundamentally alter glucose metabolism
  - Oncometabolites promote cancer
  - Antimetabolites stop cancer
  - High abundance metabolites play key cancer signaling roles
  - Metabolic disorders such as diabetes and obesity increase cancer risk substantially
  - Cachexia (a metabolic disorder) is a manifestation of cancer
  - Some of the best cancer biomarkers are metabolites
New Opportunities

• If cancer is a metabolic disease...
  – New kinds of drug targets
  – New methods for cancer prevention (diets?)
  – New approaches for early diagnosis
  – New methods for risk prediction
  – New techniques to look at cancer
  – New ways of integrating genomics with metabolomics
  – New kinds of drugs…
# Cancer Drugs That Reverse The Warburg Effect

<table>
<thead>
<tr>
<th><strong>Drug</strong></th>
<th><strong>Mechanism</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleevec</td>
<td>Inhibits Bcr-Abl, downregulates HK &amp; G6PDH</td>
</tr>
<tr>
<td>Dicholoracetate (DCA)</td>
<td>Targets and inhibits pyruvate dehydrogenase kinase</td>
</tr>
<tr>
<td>Orlistat</td>
<td>Targets and inhibits fatty acid synthase</td>
</tr>
<tr>
<td>Metformin</td>
<td>Downregulates mTOR, Activates AMPK</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Inhibits mTOR</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Inhibits glycolysis via LDH and HSF1 downregulation</td>
</tr>
</tbody>
</table>
Conclusion

Cancer as a genetic disease

• 250 oncogenes
• 700 tumor suppressors
• ~10,000-50,000 Additional mutations, CNVs or chromosomal variants in each cell
• 1 million+ different diseases

Cancer as a metabolic disease

• Aerobic glycolysis
• Glutaminolysis
• One-carbon metabolism
• 3-5 different diseases
Acknowledgements

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• Beomsoo Han
• Jeff Xia
• Lu Deng
• Rupasri Mandal
Cancer & Age

Cancer Incidence by Age 2004-2008

Rate/100,000

Age Category
What Causes Cancer?

• 5% of all cancers are inherited (germline mutations like BRCA1)
• 15-20% of all cancers arise from infectious organisms (human papilloma virus, hepatitis B/C, HIV, H. pylori)
• 75-80% arise from somatic mutations due to: ionizing radiation, pollution, chemicals, food, chronic inflammation, immunosuppression and aging
Changing Times; Changing Views

- Warburg dies in 1970
- First oncogene (Src) discovered in 1970
- Nixon declares “war on cancer” in 1971, shift in research funding to genetics
- Varmus & Bishop prove oncogene theory in 1976
- Hallmarks of cancer appears in 2000 (no mention of metabolic dysregulation)
- *From 1970-2009 the metabolic basis to cancer is largely forgotten*

Hanahan D & Wienberg RA, Cell, Jan 100(1): 57-20
Where To Next?