The Lifecycle of Biomarkers in AP – Molecular Darwinism

Jennifer L. Hunt, MD, MEd, FCAP

July 20, 2010
The Lifecycle of Biomarkers in AP –”Molecular Darwinism” will begin momentarily…….

• Don’t Forget to Register for CAP’ 10 – THE Pathologists’ Meeting – September 26 – 29, 2010 held at the Hyatt Regency Chicago!
  —Go to www.cap.org/CAP10 or call 1-800-967-4548. International attendees please call 1-847-996-5891.

• THE WEBINAR WILL BEGIN MOMENTARILY…..ENJOY!
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Associate Professor of Pathology, Harvard Medical School

Over 100 peer reviewed publications

Active member and contributor with CAP, USCAP and the Association for Molecular Pathology
Jennifer L. Hunt, MD, MEd, FCAP

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Recipient of 2006 CAP’s Foundation Lansky Award

Recipient of 2010 Arthur Purdy Stout Prize in Surgical Pathology
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Disclosure

• I have nothing to disclose.
THE

ORIGIN OF SPECIES

BY MEANS OF NATURAL SELECTION,

By CHARLES DARWIN, M.A., F.R.S., &c.

LONDON:
JOHN MURRAY, ALBEMARLE STREET.
1873.
Genetics
THE FUTURE IS NOW

New breakthroughs can cure diseases and save lives, but how much should nature be engineered?
TIME's Best Inventions of 2008

INVENTION OF THE YEAR
1. The Retail DNA Test

THE OTHER 49 BEST INVENTIONS
1. The Tesla Roadster
2. The Lunar Reconnaissance Orbiter
3. Hulu.com
4. The Large Hadron Collider
5. The Global Seed Vault

TOP 10 SCIENTIFIC DISCOVERIES
1. Large Hadron Collider
2. The North Pole — of Mars
3. Creating Life
4. China Soars into Space
5. More on the Mist
6. Brain Stands
7. The Art of Invisibility

TOP 10 ODDBALL NEWS STORIES
1. The Pregnant Man
2. Night of the Corpse Skull Bong
3. Strange Things Afoot in Vancouver
4. Virgin Shark Mother
5. She Ain't Heavy, She's My Partially Absorbed Embryonic Twin
Poster # [225]
Combined Proteomic-Transcriptomic Profiling of Laser Capture Microdissected Normal and Breast Cancer Epithelium Reveals Systematic Biochemical Network Alterations

M Imielinski, et al
March, 2010
Rudolph Virchow (1821-1902): Father of Microscopic Pathology

Kary Mullis (1983): Polymerase Chain reaction (Nobel Prize, 1993)

Carl Rokitansky (1804-1878): Father of Autopsy Pathology

Ernst Ruska & Max Knott (1931): Electron Microscope (Nobel Prize, 1986)

Questions Posed by Darwin

• Where do species come from?
• Why are there so many species?
• What contributes to long-term success as a species?
• What leads to changes in species?

Evolution
The Origin of Species: Evolution

Origination → Variation → Natural Selection → Extinction
Personalized Medicine: Definition

Medical practices targeted to individuals based on genetic differences in order to provide a tailored approach.

• Use preventive, diagnostic, and therapeutic approaches based on molecular tests and family history information

• Apply this knowledge in clinical practice for a more efficient delivery of accurate and quality healthcare through improved prevention, diagnosis, treatment, and monitoring methods.

Source: HHS Secretary Definition of Personalized Healthcare 2008
Gross Exam (1500-1800)
Biomarkers

Specific physical traits or measurable biologically produced changes in the body connected with a disease or health condition
## Biomarkers: Examples

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Assay</th>
<th>What it detects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dukes Stage</td>
<td>Histology</td>
<td>Risk stratification</td>
</tr>
<tr>
<td>Serum CEA</td>
<td>Blood test</td>
<td>Recurrent disease</td>
</tr>
<tr>
<td>EGFR protein</td>
<td>IHC</td>
<td>Therapeutic response</td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td>PCR</td>
<td>Hereditary cases</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>Sequencing</td>
<td>Nonhereditary disease</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>Sequencing</td>
<td>Therapeutic response</td>
</tr>
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Questions Posed by Darwin

• Where do species come from?
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Evolution
Questions Posed by Pathologists

• Where do biomarkers come from?
• Why are there so many biomarkers?
• What contributes to long-term success as a biomarker?
• What leads to changes in biomarkers?

Evolution
The Origin of Biomarkers: Evolution

Original Research

Clinical Application

Clinical Utility

(Extinction)
Molecular Darwinism: Biomarkers

• Natural Selection (Survival of the Fittest)
  – Microsatellite Instability
  – BRAF mutations & KRAS mutations

• Extinction
  – EGFR expression
Drivers of Biomarker Testing

- Understanding pathogenesis
- Better diagnosis
- Better prognostic information
- Better understanding of therapeutic response
Colon Cancer Biology & Prognosis

- 1990: Genetics of familial adenomatous polyposis (FAP)

- 1993: Genetics of hereditary non-polyposis colorectal carcinoma (HNPPCC)
Vogelstein Model of Carcinogenesis

APC

KRAS

p53
Mutations in Colon Cancer

- APC mutation (18%)
- p53 (26%)
- KRAS (7%)
- No Mutation (12%)

Adapted from Samowitz WS, Mol Cancer Res, 5:165, 2007
Causes of Colon Cancer

- Younger patients
- Multiple tumors (colon/other)
- Unique histology
- Family history
HNPCC: Diagnostic Testing

• Genomic DNA level
  – Gene mutations

• Tumor Protein level
  – Loss of expression of enzymes
    – MSH-2, MLH-1, MSH-6

• Tumor DNA level
  – Microsatellite analysis
  – Methylation of MLH1
DNA replication
DNA replication

Polymerase

A C G A T T A

C T A A T

T G
DNA replication
DNA Mis-Match Repair

MSH 2

MLH 1

PMS 2

MSH 3/6
Unstable Areas
Microsatellite Instability

6 repeats

4 repeats

5 repeats

7 repeats

8 repeats

9 repeats
Somatic DNA Testing

• PCR analysis of microsatellites
  – Number of repeats in normal
  – Number of repeats in tumor

• Novel sized PCR amplicons in tumor

Microsatellite Instability
## Microsatellite Instability Testing

<table>
<thead>
<tr>
<th></th>
<th>NR21</th>
<th>BAT25</th>
<th>Mono27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Tissue</td>
<td><img src="image1" alt="NR21 Normal Tissue" /></td>
<td><img src="image2" alt="BAT25 Normal Tissue" /></td>
<td><img src="image3" alt="Mono27 Normal Tissue" /></td>
</tr>
<tr>
<td>Tumor Tissue</td>
<td><img src="image4" alt="NR21 Tumor Tissue" /></td>
<td><img src="image5" alt="BAT25 Tumor Tissue" /></td>
<td><img src="image6" alt="Mono27 Tumor Tissue" /></td>
</tr>
</tbody>
</table>
Genetic of Colon Cancer

Tumor Suppressor Gene (85%)

15%

Microsatellite unstable cases (15%)

5%

HNPCC
# Genetics of Colon Cancer

<table>
<thead>
<tr>
<th></th>
<th>Tumor suppressor gene (APC)</th>
<th>DNA mismatch: hereditary</th>
<th>DNA mismatch: sporadic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microsatellite instability</strong></td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Immuno-stains</strong></td>
<td>N/A</td>
<td>hMSH2 (-)</td>
<td>hMLH1 (-)</td>
</tr>
<tr>
<td><strong>BRAF mutation</strong></td>
<td>N/A</td>
<td>Wild-type</td>
<td>Mutant (40%)</td>
</tr>
<tr>
<td><strong>hMLH1 methylation</strong></td>
<td>N/A</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>
Roles for Biomarkers in Therapeutics

• Selection for therapeutics
  – Companion diagnostics

• Predicting resistance to therapeutics
  – Markers of tumor resistance

• Monitoring of therapeutic response
  – Minimal residual disease testing
Molecular Darwinism: Biomarkers

• Natural Selection (Survival of the Fittest)
  – Microsatellite Instability
  – BRAF mutations & KRAS mutations

• Extinction
  – EGFR expression
Success of Targeted Therapy

Response

Cost
Side Effects
Epidermal Growth Factor Receptor

• Altered in many tumors
  – Protein Expression
  – Gene copy number
  – Gene sequence
# Approved Targeted Therapies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target of drug</th>
<th>Trade name</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib, Erlotinib, Cetuximab, Panitumumab</td>
<td>EGFR TKI</td>
<td>Iressa, Tarceva, Erbitux, Vectibix</td>
<td>Lung, Colon</td>
</tr>
<tr>
<td>Trastuzimab</td>
<td>Her2/neu</td>
<td>Herceptin</td>
<td>Breast</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>VEGF</td>
<td>Avastin</td>
<td>Colon</td>
</tr>
<tr>
<td>Imatinib mesylate</td>
<td>Tyrosine kinase</td>
<td>Gleevec</td>
<td>GIST, CML</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteasome</td>
<td>Velcade</td>
<td>Multiple myeloma, Mantle cell lymphoma</td>
</tr>
<tr>
<td>Rituximab</td>
<td>CD20</td>
<td>Rituxan</td>
<td>B cell lymphoma</td>
</tr>
</tbody>
</table>
Erbitux Timeline

- December, 2001: FDA negative report
- February, 2004: Literature against EGFR IHC testing
- March, 2004: FDA Approval
  - Erbitux
  - EGFR PharmDx
- Early 2005: Anticipated Completion of CA225014
- October, 2007: Completion of CA225006
- June, 2008: ASCO data: KRAS Mutation
- March, 2009: Anticipated Completion of CA225014
Internal positive control

EGFR Staining
Colon Cancer—Negative
Surrogate endpoints

- “Tumor shrinkage” by 23%
- Delayed growth (4.1 vs 1.5 mos)

Has not extended life
Dear Dr. Lee:

Unfortunately, at this time, there are no data available that demonstrate an improvement in disease-related symptoms or increased survival with Cetuximab. As a single agent, Cetuximab is indicated for the treatment of patients who are refractory to irinotecan-based chemotherapy, as indicated for the treatment of EGFR-expressing colorectal cancer.
Biomarker Extinction
Molecular Darwinism: Biomarkers

• Natural Selection (Survival of the Fittest)
  — Microsatellite Instability
  — **BRAF mutations & KRAS mutations**

• Extinction
  — EGFR expression
Oncogene Mutations

- KRAS mutations
- BRAF mutations
Roles for Biomarkers in Therapeutics

• Selection for therapeutics
  – Companion diagnostics

• Predicting resistance to therapeutics
  – Markers of tumor resistance

• Monitoring of therapeutic response
  – Minimal residual disease testing
Cost Per Month

- Gleevec: $3,000
- Herceptin: $2,800
- Rituxan: $12,000
- Iressa: $1,800
- Tarceva: $2,700
- Erbitux: $9,600
- Vectibix: $8,000

75% of personal bankruptcy is caused by a catastrophic illness

Himmelstein DU. 24(1), 2005
KRAS Mutations in Colon Cancer

Liu, in preparation
Bokemeyer C, Abstract 4000, ASCO 2008
Cervantes A, Abstract 4129, ASCO 2008
Merlin JL, Abstract 4126, ASCO 2008
DiFiore, Abstract 4035, ASCO 2008
Van Cutsem, Abstract 2, ASCO 2008
KRAS and BRAF Mutations Rates

<table>
<thead>
<tr>
<th>Mutation rate</th>
<th>CCF rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>10%</td>
</tr>
<tr>
<td>KRAS</td>
<td>40%</td>
</tr>
<tr>
<td>BRAF</td>
<td>10%</td>
</tr>
<tr>
<td>KRAS</td>
<td>9%</td>
</tr>
<tr>
<td>BRAF</td>
<td>29%</td>
</tr>
<tr>
<td>KRAS</td>
<td>29%</td>
</tr>
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</table>
KRAS and Response Rate

Bokemeyer C, Abstract 4000, ASCO 2008
Cervantes A, Abstract 4129, ASCO 2008
Merlin JL, Abstract 4126, ASCO 2008
BRAF and Response Rate

![Graph showing response rates for mutant and wildtype BRAF.]

Di Nicolantonio F. J Clin Oncol, 26:5705, 2008
Detection of Oncogene Mutations

• Gene sequencing
  – Traditional sequencing
  – Pyrosequencing

• Allele specific PCR

• Kit based testing
  – Mutector®
Sequencing: The Gold Standard

Template DNA

PCR with primers

PCR Product

Sequencing reaction

Sequence
BRAF Mutation
KRAS Codons 12 & 13
Questions Posed by Pathologists

• Where do biomarkers come from?
• Why are there so many biomarkers?
• What contributes to long-term success as a biomarker?
• What leads to changes in biomarkers?
Questions Posed by Pathologists

• Where do **biomarkers** come from?
• Why are there so many **biomarkers**?
• What contributes to long-term success as a **biomarker**?
• What leads to changes in **biomarkers**?

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  – Speaker: Guillermo (Gary) J. Tearney, MD, PhD

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  – Personalized Pathology: PHC in the General Pathology Practice
  – Introduction to the Medical Home
  – Personalized Medicine: Framing the Issues for Pathology
  – Clinical Requests for Molecular Tests