Clinical Implications of Epigenetics Research

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Vice Chair of Academic Affairs
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Epigenetics: Our Software
What is Epigenetics?

- Heritable changes in gene expression not due to changes in the DNA sequence
  - DNA methylation
  - Chromatin structure or histone code

- Role in normal and pathological processes, such as aging, mental health, and cancer, among others.
DNA Methylation changes in Neoplasia

Normal

Cancer
Epigenetic changes in Neoplasia

**Normal**

- MeK4
- AcK9

**Cancer**

- MeK27
- MeK9
- ??
Epigenetic changes in Neoplasia

Normal

Aging
Inflammation

Cancer

HATs
HDemethylases

HDACs
HMTases

Ahuja et. al. Cancer Res. 1998
Issa et. al Cancer Res 2001
Cancer Epigenetics: Applications

Repressor proteins (MBD, HDAC)

DNA

Promoter Gene

Cancer Marker for Molecular Staging

Early Detection

Prognostic
And Predictive Biomarkers

Epigenetic Therapy

Reversal of Gene Silencing
Colon Cancer Functional Methylome

Yellow spots indicate genes from DKO cells with 2 fold changes and above. Green spots indicate experimentally verified genes. Red spots indicate those that did not verify. Blue spots indicate the location of the 11 guide genes used in this study.

• Each colon cancer has ~150 to 450 methylated genes/tumor

Schuebel et. al. PLoS Genet. 2007
Early Detection of Cancers

Methylation of *TFPI2* in Stool DNA: A Potential Novel Biomarker for the Detection of Colorectal Cancer

**TFPI2 Stool Methylation:** Sensitivity: 76-89%; Specificity: 79-93%

Glockner *et al.*, Cancer Res 2009
Next Generation Stool DNA Testing for Detection of Colorectal Neoplasia

Early Marker Evaluation

David Ahquist*, Hongchi Zou†, Michael Domanico§, Douglas Mahoney∥, Stephen Thibodeau∥, Graham Lidgard∥

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester MN, Exact Sciences, Madison WI

AACR CRC Conference 2010
Pancreas Cancer - A Lethal Disease

Pancreas Cancer is the fourth leading cause of cancer death in the USA with 45,220 estimated new cases and 38,460 estimated deaths in 2013.
Pancreas Cancer-A Global Problem

• 13\textsuperscript{th} most common cause worldwide with 280,000 people diagnosed each year; 8\textsuperscript{th} most common cause of cancer death

• 85\% of patients are unresectable;

• 75\% of patients die with a year.
Changing the Outcome

No improvement in survival over 40 years

Early Pancreas Cancer has no SYMPTOMS

*Only Way to Improve Survival is Early Detection when Cancer is in its earliest stage*
Changing the Outcome of Pancreas Cancer

NO SCREENING TEST FOR PANCREAS CANCER!

Goal - To develop a simple, screening blood test for detection of pancreas cancer
Proposal

• To develop a simple blood test using a genome screen for screening high risk patients

• Test can be repeated at low cost annually

• High patient acceptance of blood based screening
Gene Selection

Methylation Analysis

Mutation Analysis

2,607 Genes → 10 Candidate Genes → 1,327 Genes

Identifying Candidates

Validation in Primary Tissues

Markers detect early stage, curable cancers!

Table 3. Comparison of BNC1 and ADAMTS1 methylation and CA 19-9

<table>
<thead>
<tr>
<th>Stage</th>
<th>BNC1</th>
<th>ADAMTS1</th>
<th>ADAMTS1 and BNC1 combined</th>
<th>CA 19-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>PanIN (n = 20)</td>
<td>70%</td>
<td>25%</td>
<td>75%</td>
<td>20%</td>
</tr>
<tr>
<td>Stage I (n = 38)</td>
<td>97%</td>
<td>63%</td>
<td>97%</td>
<td>52%</td>
</tr>
<tr>
<td>Stage II (n = 78)</td>
<td>96%</td>
<td>82%</td>
<td>96%</td>
<td>73.7%</td>
</tr>
<tr>
<td>Stages III and IV</td>
<td>100%</td>
<td>57%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

NOTE: The rates of methylation for either BNC1, ADAMTS1, or BNC1 and ADAMTS1 combined were identified for PanIN and invasive pancreatic cancers, stage I, stage II, and stages III and IV. Any methylation was considered to be a positive test. CA 19-9 levels were derived from patients who had evaluation of a CA 19-9 level before surgery. Any value 37 U/mL and greater was considered to be positive.

MOB: Methylation On Beads

Fig. 1. Pre-PCR DNA yield from serum for the MOB method and the conventional PC/bisulfite conversion method.
Yields have been normalized for an input of 25 μL of serum.

Bailey et al; Clin Chem: 2010
## Testing in Serum Samples

<table>
<thead>
<tr>
<th></th>
<th>Estimated Value (%)</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>79</td>
<td>68-86</td>
</tr>
<tr>
<td>Specificity</td>
<td>89</td>
<td>69-97</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>59</td>
<td>48-69</td>
</tr>
<tr>
<td>Specificity</td>
<td>92</td>
<td>74-98</td>
</tr>
<tr>
<td>Overall Sensitivity</td>
<td>83.7</td>
<td>74-90</td>
</tr>
<tr>
<td>Overall Specificity</td>
<td>84.6</td>
<td>64-95</td>
</tr>
</tbody>
</table>

Novel Methylation Biomarker Panel for the Early Detection of Pancreatic Cancer

Joo Mi Yi, Angela A. Guzzetta, Vasudev J. Bailey, Stephanie R Downing, Leander Van Neste, Katherine B. Chiappinelli, Brian P. Keeley, Alejandro Stark, Alexander Herrera, Christopher Wolfgang, Emmanuel P. Pappou, Christine A. Iacobuzio-Donahue, Michael G. Goggins, James G. Herman, Tza-Huei Wang, Stephen B. Baylin, and Nita Ahuja

Tools & Resources

6 Serious Medical Symptoms
The Costs of Cancer
Top Cancer-Fighting Foods
Maryland Health Connection
Pancreatic Cancer, In Pictures
Swayze's Widow Shares Story

Blood Test for Pancreatic Cancer Shows Promise

But the screen is meant only for people already at high risk for the deadly illness, experts say

By Alan Mozes
HealthDay Reporter

FRIDAY, Oct. 25 (HealthDay News) -- Pancreatic cancer is one of the most lethal tumor types because it's too often diagnosed in a later, advanced stage. But a new study suggests that a simple blood test might help spot the disease earlier.

The study is described as small and preliminary, and investigators cautioned that the initial findings will need to be confirmed in larger trials.

The findings of their research, if confirmed, say, could be an important step in reducing mortality from the cancer, which has an overall five-year survival rate of less than 5 percent and has seen few improvements in survival over the last three decades.

"We have mammograms to screen for breast cancer and colonoscopies for colon cancer but we have had nothing to help us screen for pancreatic cancer," says Nita Ahuja, M.D., an associate professor of surgery, oncology and urology at the Johns Hopkins University School of Medicine and leader of the study described online this month in the journal Clinical Cancer Research. "While far from perfect, we think we have found an early detection marker for pancreatic cancer that may allow us to locate and attack the disease at a much earlier stage than we usually do."

For their study, Ahuja and her colleagues were able to identify two genes, BNC1 and ADAMTS1, which together were detectable in 81 percent of blood samples from 42 people with early stage pancreatic cancer, but not in patients without the disease or in patients with a history of pancreatitis, a risk factor for pancreatic cancer. By contrast, the commonly used PSA antigen test for prostate cancer only picks up about 20 percent of prostate cancers.
Next Steps

• Test needs validation in larger cohort of patients with cancers and normal populations

• Adapt test for CLIA application
Cancer Epigenetics: Applications

- Reversal of Gene Silencing
- Epigenetic Therapy
- Cancer Marker for Molecular Staging
- Early Detection
- Prognostic and Predictive Biomarkers
- Reversal of Gene Silencing

Diagram:
- DNA
- Promoter
- Gene
- Repressor proteins (MBD, HDAC)

Prognostic

Epigenetic Therapy
Genomic Landscapes of Colon and Breast Cancers

Complexity of cancers - multiple mutations

How do we develop then better targeted therapies?

Wood et al, Science. 2007;318:1108-13
Integration of genomic and epigenomic landscapes

- Epigenetic changes are more frequent than genetic events
- Common targets

Yi et al., Clin Can Res 2011
Prognostic Markers: Colon Cancer

Yi et al., Clin Can Res 2011
Carmona and Esteller Clin Can Res 2011
Biomarkers for detection and prognosis of breast cancer identified by a functional hypermethylome screen

Jana Jeschke,1,3,5 Leander Van Neste,4,5,6 Sabine C. Glöckner,1,6,7 Mashaal Dhir,1 Marilia Freitas Calmon,1,2 Valérie Deregowski,5 Wim Van Criekinge,4,5 Ilse Vlassenbroeck,5 Alexander Koch,4 Timothy A. Chan,7 Leslie Cope,6 Craig M. Hooker,2 Kornel E. Schuebel,2 Edward Gabrielson,9 Andreas Winterpacht,3 Stephen B. Baylin,2 James G. Herman2,*, and Nita Ahuja1,2,*
Prognostic Biomarkers

Jeschke et al., Epigenetics 2012
Epigenetics and Reprogramming

Epigenetic-mediated gene silencing in cancer

DNMT Inhibitors
- Azacitidine
- Decitabine
- Zebularine

HDAC Inhibitors
- Sodium phenylbutyrate
- Valproic acid
- Vorinostat (SAHA)
- Romidepsin (FK228)
- Entinostat (SNDX-275)
- Panobinostat (LBH589)
- Belinostat (PXD101)

Reprogram cellular epigenetic alterations to inhibit cancer

Tumor suppressor genes reexpression
- Lineage commitment
- Immunomodulation
- Modulate signaling pathways (Wnt, TGFβ, etc)
- Programmed cell death

Tsai and Baylin Cell Research 2011
DNA Methylation: A REVERSIBLE MODIFICATION

VIDAZA (5-Azacitidine or 5-AZA) 
DACOGEN (5-aza-2'-deoxycytidine 
or DAC)

FDA Approved Therapy for treatment of MDS, precursor of leukemia
Transient Low Doses of DNA-Demethylating Agents Exert Durable Antitumor Effects on Hematological and Epithelial Tumor Cells

Chen-Tsai et al. Cancer Cell 21; 2012
Epigenetic Therapy Clinical Trials

Jean-Pierre Issa
Charles Rudin
Ros Juergens
Malcolm Brock
Nita Ahuja
Nilo Azad
Roisin Connolly, M.B.

Anthony El-Khoueiry, Casey O’Connell, Barbara Gitlitz, Debu Tripathy

Leukemia
Breast, Lung, & Colon

Mayo-Consortium - Washington Univ, Rochester Univ, Mayo Clinic
Breast, & Lung

Nancy Davidson
Rachel Jankowitz, M.D.
MC084B – Phase II Study of Azacitidine and Entinostat in Patients with Metastatic Colorectal Cancer

- **Primary Objective:**
  - To determine the preliminary efficacy via RECIST response rate of the combination of 5-azacitidine and Entinostat in patients with metastatic colorectal cancer

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Azad et al ASCO 2013
## Study Enrollment: 39/40 Enrolled

<table>
<thead>
<tr>
<th>Cohort</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number treated/enrolled</td>
<td>23/24</td>
<td>21/23</td>
</tr>
<tr>
<td>Median Age (range)</td>
<td>57 (28-75)</td>
<td>63 (32-75)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- White</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>- Black</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>- Other</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Prior therapies (all settings)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 2</td>
<td>8.3%</td>
<td>47.8%</td>
</tr>
<tr>
<td>- 3</td>
<td>25%</td>
<td>21.7%</td>
</tr>
<tr>
<td>- 4</td>
<td>16.7%</td>
<td>17.4%</td>
</tr>
<tr>
<td>- 5</td>
<td>29.2%</td>
<td>4.3%</td>
</tr>
<tr>
<td>- 6</td>
<td>4.2%</td>
<td>8.7%</td>
</tr>
<tr>
<td>- 7</td>
<td>8.3%</td>
<td></td>
</tr>
<tr>
<td>- 9</td>
<td>8.3%</td>
<td></td>
</tr>
<tr>
<td>Location of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lung</td>
<td>79.2%</td>
<td>73%</td>
</tr>
<tr>
<td>- Liver</td>
<td>75%</td>
<td>78%</td>
</tr>
<tr>
<td>- Abdominal</td>
<td>33.3%</td>
<td>52%</td>
</tr>
</tbody>
</table>

Azad et al ASCO 2013
MC084B – Amended Trial Design

• Eligibility Criteria Modifications:

– Failed <2 prior chemotherapy regimens in the metastatic setting if KRAS mutation positive.   OR

– 3 prior regimens in the metastatic setting if KRAS wild-type (with one of those regimens being an anti-EGFR).

– Liver disease burden limited to no more than 30% of total liver volume as assessed through liver volumetric assessment on scan by central radiologist review.

Azad et al ASCO 2013
MC084B – Phase II Study of Azacitidine and Entinostat in Patients with Metastatic Colorectal Cancer

**Cohort 1 (n=23)**
- 26% treated alive 12 mo

**Cohort 2 (n=21)**
- 38% treated alive 12 mo

Subsequent therapies: response still pending
- **Cohort 1:** 4 patient
- **Cohort 2:** 13 patients

Azad et al ASCO 2013
Historical Perspective

Cetuximab vs BSC

Van Cutsem E et al. JCO 2007;25:1658-1664

Jonker et al. NEJM 2007; 257;20 2040-2048

Axel Grothey et al. Lancet 2013. 381; 9863: 303 - 312

Panitumumab vs BSC

Van Cutsem E et al. JCO 2007;25:1658-1664
Epigenetic Therapy: Current Concepts

- Mandatory serial biopsies and serum samples are feasible and successfully obtained in a multicenter trial.

- Drugs are well tolerated in combination in mCRC.

- In metastatic colon cancer, epigenetic therapy effects, like molecularly targeted agents, do not result in tumor shrinkage.
  - Appropriate clinical endpoints are key to truly evaluate these agents.
  - Potential endpoints: survival, response rate to subsequent chemotherapies.
Epigenetic Therapy: Current Concepts

- Median overall survival and 12 month overall survival in cohort 2 is better than historical controls – may be hampered by complete lack of subsequent therapy options

- Next steps
  - Continued molecular analysis for expression and methylation analysis
  - Clinical trial of epigenetic priming based on preclinical work - LOI accepted and protocol beginning review process
A Phase 1/randomized Phase II study of SGI-110+Irinotecan vs. regorafenib in mCRC

**Phase 1:**
- Standard 3+3 Design
- 28 day cycles
- Paired tumor biopsies pre- and post-treatment
- Research Blood

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>SGI-110 Dose</th>
<th>IRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>30 mg/m2 d1-5</td>
<td>125 mg/m2 d8 and 15</td>
</tr>
<tr>
<td>1</td>
<td>45 mg/m2 d1-5</td>
<td>125 mg/m2 d8 and 15</td>
</tr>
<tr>
<td>2</td>
<td>60 mg/m2 d1-5</td>
<td>125 mg/m2 d8 and 15</td>
</tr>
<tr>
<td>3</td>
<td>SGI-110 MTD dose</td>
<td>125 mg/m2 d8 and 15 cycle 1 and d1,8, and 15 of subsequent cycles</td>
</tr>
</tbody>
</table>

**Phase II:**
- Patients randomized 2:1 to SGI-110+IRI MTD vs Regorafenib
- 4 m PFS is primary endpoint
- Crossover design (compromises OS, however)
- Patients of SGI-110+IRI allowed to stay on study past first progression if clinically stable
Other Ongoing Clinical Trials

• A multi-institutional open label, trial evaluating the efficacy of Gemcitabine and Docetaxel in patients with relapsed or refractory metastatic colorectal adenocarcinoma with methylated CHFR and/or microsatellite instability phenotype

• J11102: A Phase I trial of oral 5-azacitidine in combination with romidepsin in advanced solid tumors, with an expansion cohort in non-small cell lung cancer

• A Phase II trial to improve outcomes in patients with resected pancreatic adenocarcinoma at high risk for recurrence using epigenetic therapy
Future Directions: Epigenetics Priming

Chemotherapy Response

Immune response

Baylin SB Nat Med 2011
Summary

- Epigenetic changes are common in cancer
- DNA methylation marks can be used for early detection of many cancers
- Methylation profiles can inform us about prognostic potential of cancers
- Epigenetic therapy may be given safely and has shown preliminary promise in solid tumors
- Future directions aimed at using epigenetic priming to reverse chemoresponse and immune evasion in solid tumors
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