A Human Gene and Transcription to RNA and Translation to Protein

Gene (DNA)

Transcript (RNA)

mRNA (RNA)

Protein
Gene Regulatory Mechanisms

• Transcriptional Mechanisms
  – Type of promoters & RNA polymerases
  – Control of transcription by transcription factors and TFBS

• RNA processing
  – 5’ capping & 3’ poly-adenylation
  – RNA degradation rates
  – Splicing and alternative splicing

• Translational Mechanisms
  – Micro RNAs (miRNAs) inhibit translation and degrade mRNA
  – Silencer RNAs (siRNAs or RNAi) degrading mRNA

• Epigenetic Mechanisms
  – DNA methylation
  – Histone modifications: acetylation, methylation, phosphorylation, etc.
  – Chromatin remodeling
Eukaryotic Gene Structure
Multiple Enhancer Sequences
Gene Expression Regulatory Network
Human Mitotic Chromosome
DNA in a Human Chromosome
DNA in a Human Chromosome
Three Levels of Folding of DNA in Chromatin
Folding of DNA in Nucleosome
Interferon-Beta Gene Enhancer in Macrophage Cells
Enhanceosome: Exploring the Structure

Enhanceosome: Integrating the Signal


HAT = Histone Acetyl Transferase

Integrating the Signal

Once the transcription factors bind to the different sites in the enhancer DNA sequence, the signal must somehow be sensed and used to activate the gene. In many cases, this is performed using CREB-binding protein or the similar protein p300. This protein is composed of many connected domains, (PDB entries 18c, 1kdx, 1jsp, 3biy, 2ka6 and 1khh), which bind to different proteins in the assembled enhanceosome. Then, a large domain in the center acts as a histone acetyltransferase, modifying histones in nucleosomes and causing them to disassemble and reveal the gene. In the interferon-b gene, a nucleosome normally covers the start site of transcription, blocking transcription. Assembly of the enhanceosome leads to removal of this nucleosome, allowing the gene to be expressed.
Repressor Bound to Silencer Sites
Hematopoiesis

http://www.biocarta.com/pathfiles/h_stemPathway.asp
Cytokine Network

http://www.biocarta.com/pathfiles/h_cytokinePathway.asp
Interferon Signaling
DNA Microarrays & DNA Chips
Accelerate Genetic Analysis

• Parallel analyses
  – Analyze entire genomes instead of single genes
  – Analyze expression of entire genome
  – Analyze genetic polymorphisms (SNPs)
• Miniaturization
• Automation
Diagnosis Using DNA Arrays

http://www.affymetrix.com/
Affymetrix Arrays
http://www.affymetrix.com/

- High-density grid of DNA sequences.
- Any collection of 25mers (1,200,000) can be synthesized in 100 steps.
- The location and identity of each sequence on the glass surface is known.
Light Directed Oligonucleotide Synthesis

http://www.youtube.com/watch?v=ui4BOtwJEXs&feature=related
Automated DNA Chip Synthesis

http://www.affymetrix.com/
Photolithography Masks

http://www.affymetrix.com/
Hybridization & Detection

before hybridization

labeled target

after hybridization
Human Gene Expression Signatures

- T Cells Signaling
- DNA Damage
- Fibroblast Stimulation
- B Cells Signaling
- CMV Infection
- Anoxia
- Polio Infection
- Cell Signaling IL4
- Growth Hormone
Microarrayer in Pat Brown’s Lab
http://cmgm.stanford.edu/pbrown/
High Precision DNA Printing
Mechanical Spotting Microarrays
http://www.arrayit.com/

Bubble Pin Technology
DNA Microarray cDNA Labeling
Breast Cancers Classified by 451 Gene Expression Assays

Sorlie et al., PNAS 98, 10869-10874.
Breast Cancers Classified by 451 Gene Expression Assays

Sorlie et al., PNAS 98, 10869-10874.
ERB-B2 in Signal Transduction & Oncology

http://www.biocarta.com$pathfiles$h_her2Pathway.asp
Herceptin binds to HER2
Blocking Cell Growth

Herceptin = anti-HER2
Fab Fragment

HER2 (Erb-2)
Human Epidermal
Growth Factor
Receptor
Regulation of tumor suppression by PML
http://www.biocarta.com/pathfiles/h_pmlPathway.asp
Acute Promyelocytic Leukemia

Tumor cDNA + Retinoic Acid (24 hr)

(Doug Ross & Pat Brown)
Examples of the genetic alterations leading to four representative cancer types are shown. Each gene symbol denotes a pathway. For example, APC denotes the pathway regulated by APC. A “mutation” in a pathway can be achieved by genetic or epigenetic inactivation of both alleles of a tumor-suppressor gene or by genetic activation of an oncogene in that pathway. Pathogenic strains of human papillomavirus initiate the breakthrough phase by disabling both the TP53 and RB pathways.
Targeting Chronic Myeloid Leukemia (CML) Cells with a Double Whammy.

Predicting Tissue of Origin for Cancers of Unknown Primary
PathWork Oncology Suite: Site of Origin for Solid Metastatic Tumors

- 1844 tumors tested one at a time versus all 18 tissues of origin
- Tests chosen based on the NCCN guidelines for treatment
- Retrospective study on well characterized patient samples
- Uses PathChip (functionally similar to Affymetrix HU-133A GeneChip)
  - 604 specimens used for training
  - 636 specimens used for test
  - 604 specimens in reserve for final validation
- Reproducibility from lab to lab
- Performance based on sensitivity (> 70%) & accuracy (> 95%)
# Datasets

<table>
<thead>
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<th>Class</th>
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### DEMONSTRATION EXAMPLE REPORT

**PATHWORK ONCOLOGY SUITE: SITE OF ORIGIN**

**GENE EXPRESSION-BASED MOLECULAR DIAGNOSTIC**

<table>
<thead>
<tr>
<th>SITE OF ORIGIN</th>
<th>PPV</th>
<th>NPV</th>
<th>SIMILARITY SCORE</th>
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<td>99%</td>
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<tr>
<td>Squamous</td>
<td>48%</td>
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<tr>
<td>Thyroid</td>
<td>95%</td>
<td>100%</td>
<td>-98</td>
</tr>
<tr>
<td>Bladder</td>
<td>67%</td>
<td>99%</td>
<td>-99</td>
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<tr>
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<td>100%</td>
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<td>Germ Line</td>
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<td>100%</td>
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<tr>
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<td>100%</td>
<td>-100</td>
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<tr>
<td>Melanoma</td>
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<td>100%</td>
<td>-100</td>
</tr>
<tr>
<td>Soft Tissue-Sarcoma</td>
<td>39%</td>
<td>99%</td>
<td>-100</td>
</tr>
</tbody>
</table>

**SIMILARITY SCORE**

**NEGATIVE**

**POSITIVE**

**KEY**

Sample Site: Liver  
Primary Site: CO  
Histology Type: Adenocarcinoma  
Sample Description: PARTIAL HEPATIC/MESENTERIC ADENOCARCINOMA PRIMARY IN COLON. STATUS POST CHEMOTHERAPY.

For use by CLIA-certified clinical laboratories only. The clinical interpretation of the results should be made in context of the patient's clinical history and other diagnostic tests performed by a qualified individual.

Advanced Pathology Labs  
123 Diagnostic Drive, Multiplex, CA 92612  
(800) 555-1212  

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# Demonstration Example Report

**Pathwork Oncology Suite: Site of Origin**

**Gene Expression-Based Molecular Diagnostic**

**Patient ID:** Lurn1878  
**Specimen Source:** Lung  
**Medical Record:** Order Pathologist  
**Case Number:** Tissue Path Inc.  
**PathWork Accession:** SO 00030  
**Date/Time Processed:** 25 JU 2005 11:24 PDT  
**Version:** SO012.8

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<th>Positives</th>
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<td>100%</td>
<td>-100</td>
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<td>39%</td>
<td>99%</td>
<td>-39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Non-Small Cell Lung Cancer**

**KEY**

- **Sample Site:** Lung  
- **Primary Site:** CO  
- **Histology:** Adenocarcinoma  
- **Sample Description:** LULL LUNG WEDGE BIOPSY, METASTATIC ADENOCARCINOMA C/W COLONIC PRIMARY DIAGNOSED 7 YEARS PRIOR; STAGING AT TIME OF ORIGINAL DIAGNOSIS UNKNOWN.

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Cancer Genetics Inc.
http://www.cancergenetics.com/

TOO®
Tissue of Origin®
Test Available

CGI Now Offers FDA-cleared Tissue of Origin® Test
The Tissue of Origin® test, formally a Pathwork test, is a microarray-based gene expression test that aids in identifying challenging tumors, including metastatic, poorly differentiated, and undifferentiated cancers. It's the only FDA-cleared test of its type, and is a Medicare-reimbursed test. Learn More >>
T cells need 2 signals for activation

- Signal 1: Antigen recognition
- Signal 2: Co-stimulation

- There are positive and negative second signals

Gordon Freeman, Science Webinar on Immune Checkpoints
Activating and Inhibitory T Cell Signals

Philip Gotwals, Science Webinar on Immune Checkpoints
Anti CTLA-4 Antibody Stimulates Immune Response

CTLA-4 Blockade Enhances Tumor-Specific Immune Responses

Attenuated or Terminated Proliferation

Unrestrained Proliferation

IL-2

James Allison, Science Webinar on Immune Checkpoints
Anti-CTLA-4 Induces Colon Cancer Tumor Regression

James Allison, Science Webinar on Immune Checkpoints
Ipilimumab
(Medarex, Bristol-Myers Squibb)

Fully human antibody to CTLA-4
>50,000 patients treated to date:

Objective responses in many tumor types, including melanoma, prostate, kidney, bladder, ovarian & lung cancer, etc.

Adverse events (colitis, hepatitis, hypophysitis, etc.) serious but generally manageable

James Allison, Science Webinar on Immune Checkpoints
Anti PD-1 Also Reactivates T Cells

T Cell

Increased cytokines

IFN-γ

antibody drug

PD-L1

TCR

MHC

Tumor cell

Increased killing

Gordon Freeman, Science Webinar on Immune Checkpoints
Activating and Inhibitory T Cell Signals

Philip Gotwals, Science Webinar on Immune Checkpoints
FDA Approval of Immune Checkpoint Antibodies

- 2011 Ipilimumab (BMS) – Melanoma
- 2014 Pembrolizumab (Merck) – Melanoma
- 2014 Nivolumab (BMS) – Melanoma
- 2015 Nivolumab (BMS) – Lung
- 2015 Ipilimumab + Nivolumab (BMS) – Melanoma
- 2015 Pembrolizumab (Merck) – Lung
- 2015 Ipilimumab (BMS) – Adjuvant melanoma
- 2015 Nivolumab (BMS) – Renal cell carcinoma
On Cancer

Understanding Jimmy Carter’s Surprise Cancer Turnaround: A Conversation with Jedd Wolchok

By Matthew Tontonoz on Wednesday, December 9, 2015

Former President Jimmy Carter announced this week that he is “cancer free” after receiving treatment for advanced melanoma. Photo Credit: The Carter Center.
The Emperor of All Maladies
A Biography of Cancer

Siddhartha Mukherjee

"A compulsively readable, surprisingly uplifting, and vivid tale. Thrilling."
—O. The Oprah Magazine
KEN BURNS PRESENTS

CANCER

THE EMPEROR OF ALL MALADIES

A FILM BY BARAK GOODMAN

BASED ON THE BOOK THE EMPEROR OF ALL MALADIES: A BIOGRAPHY OF CANCER
BY SIDDHARTHA MUKHERJEE

EXPLORE THE FILM