The Molecular Pathogenesis of Cancer

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Breast Cancer Facts

• 180,000 new breast cancer cases are diagnosed each year in the United States

• Breast cancer is the leading cause of cancer death among women ages 35 to 54

• Breast cancer is the second-leading cause of cancer death in women after lung cancer

Further research is needed to identify factors that cause breast cancer and to improve the detection and treatment of this disease.
Challenge:
To Identify the Achilles’ Heel of Tumor Cells

Designer therapies

Tumor heterogeneity, genetic instability, and the complexity of tumor interactions with cells in their environment complicate the task of identifying these sensitivities.
Invasive Ductal Carcinoma
High Grade

To develop therapies designed to target the events that drive tumor initiation and progression of individual tumors, it is important to understand what processes/proteins/genes are responsible for events associated with the initiation and progression of the tumor.
Different Stages and Grades of Breast Tumors

- Normal Acini
- Atypical Ductal Hyperplasia
- Carcinoma in situ
- Invasive Carcinoma

Tumor images from Dennis Sgroi-MGH
Breast tissue is Composed of Ducts and Glands Where Cancer Originates
Initiation and Progression of Breast Cancer

Normal Gland → Hyperplasia → Carcinoma in situ → Invasive Carcinoma

Carcinoma in situ
Invasive Carcinoma

Hyperplasia

Normal Gland

Carcinoma in situ
What Causes Breast Cells to Change their Behavior?

Different types of breast tumors contain mutations in different genes.
Most Cancers Contain Gross Chromosomal Aberrations

Amplifications

Deletions

Comparative Genome Hybridization
Identification of Mutations in Cellular Genes

Abnormal/Mutant Protein -
- Lower or greater activity
- Unregulated activity

Mutation in regulatory sequences
- Increased protein production
- Decreased protein production
Proteins control all aspects of cell function:

• they are the basic building blocks for most components of the cell -
• they also catalysts for regulation of cell behavior
Some proteins tell promote cells to proliferate.

Other proteins halt division or tell cells to die.

These are over-produced or mutated causing them to always ON in cancer.

These are lost or underproduced in cancer.
Initiation and Progression of Breast Cancer

- Normal Gland
- Hyperplasia
- Carcinoma in situ
- Invasive Carcinoma

- Loss of growth suppressor
- Amplification of survival protein
- Induction of an invasive protein

Breast Cancer Genes

- Amplification of survival protein
- Induction of invasive protein

Integrin Signaling

- Lamellipodia
- Jnk
- Vav
- Akt
- Cbl
- Rac
- Erk
Initially looked at individual genes/proteins that were known to be involved in regulating cell growth or that were associated with hereditary breast cancer.

Multiple proteins were identified as being altered in human breast tumors

<table>
<thead>
<tr>
<th>Protein</th>
<th>Alteration</th>
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<tbody>
<tr>
<td>p53</td>
<td>lost/mutated</td>
</tr>
<tr>
<td>BRCA1</td>
<td>lost/mutated</td>
</tr>
<tr>
<td>BRCA2</td>
<td>lost/mutated</td>
</tr>
<tr>
<td>Cyclin D</td>
<td>increased production</td>
</tr>
<tr>
<td>Myc</td>
<td>increased production</td>
</tr>
<tr>
<td>HER-2</td>
<td>gene amplified</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>misregulated</td>
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</tbody>
</table>
“Genomics” Approaches

Normal and malignant specimen procurement and collection of clinical data

Data storage and analysis
Hierarchical or k-means clustering
Hundreds of proteins show decreased or increased production in breast tumors.

Each tumor varies a great deal, but some patterns are emerging where different sets of genes are found to vary together.

Would like to correlate a specific “signature” of genes with subtypes of tumors.

Most importantly, need to find out how to use this information to prevent/treat disease.
Which of the 100’s of Altered Genes are Involved in Cancer?

What cellular pathways and processes do they control?
Models to Investigate the Biological Activity of Genes Altered in Cancer

Animal models

Tissue culture models

Suspended in agar
Model of Breast Epithelial Acini

http://www.udel.edu/Biology/Wags/histopage/empage/efr/efr3.gif
Cell Culture Model of Epithelial Acini

Matrigel - Collagen/laminin/entactin

Brightfield image

Cross-section-nuclear stain

http://www.udel.edu/Biology/Wags/histopage/empage/efr/efr3.gif
Reconstruction of Tumor Phenotype

Genes implicated in breast cancer

Examine the effects of expression/activation of biological activities of proteins known or suspected to be involved in human breast cancer

Define the cellular pathways required for induction of phenotypic changes
## Breast Cancer 1000 Project

| ANSA                  | Ap14 | Bcl2L1 | BIK | Casp2 | Crkl | Csmk1E3 | Max | CDK N1A, 2A, 1B | VEGF (X3) | Taifi30 | Mad | Cyclin D1,2,3 | Wnt10B | RAB9 | Rad51 | Wnt7A | Wnt2 | TGFβ receptor II | Fli1 | Gro2 | Hck | IGFbP3 | IGFbP7 | IGF1b | Jun | Lcn2 | Mac25 | Mafk | UGB | OXCT | PDGFB | RAB31 | Wnt5a | Cdc37 | Rad51L | Rap2b | S100A4 | SnCG | CPOCK | SPP1 | TDPX1 | Thra | Ube2b | Eif4e | Eif3S6 | Dad1 |
|-----------------------|------|--------|-----|-------|------|---------|-----|--------------|----------|--------|-----|---------------|--------|------|-------|-------|-----|------------------|------|------|-----|--------|--------|-------|------|------|-------|------|------|------|-------|-------|------|------|------|------|------|------|------|------|
| Ras                   | HGF  | RARγ   | Raf1| ITPKA | Mmp1 | Hdac1, | Pparγ | Tgfbr2 | Mmt-Mmp | Esr1  | Ptrl | Fzd1 | CST6 | Bad   | BAG1 | RAD54L |     |       |     |       |       |       |     |     |     |       |       |       |     |     |     |       |       |     |     |     |     |     |     |     |     |
|                       |      |        |     |       |      |        |       |       |         |       |      |     |     |       |       |     |       |     |       |     |       |       |       |     |     |     |       |       |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

*Josh LaBaer/Ed Harlow et al/HIP*
Laboratory Cancer Model

Normal structure

Hyperplastic structure

cyclinD

Filled structure

IGF-1R
HER2/Neu in Breast Cancer

HER2 Gene Amplified or overproduced in 25% of human tumors

Proliferation/Survival
HER2 Induces Structures that Resemble Human Tumors
Two Paths Leading to Filling of the Lumen

- Cyclin D
- E7 loss of Rb/p21

Bcl-2

ErbB2
Pathways to Luminal Filling

- IGF-1R
- Bcl-2/Bcl-xl
- cyclinD
- HPV-E7
- ErbB2
- CSF-1R
- V-Src
- IGF-1R
Conversion from Carcinoma-in-situ to Invasive Structure

Invasive Carcinoma

ErbB2

ErbB2 + TGFβ
Ran Expression Induces an Invasive Phenotype in CSF-1R Structures

CSF-1 + wt CSF-1R

Ran +
CSF-1 + wt CSF-1R
A Cure For Cancer - The Challenge

Because of many alterations in breast cancer and the complexity of the disease, it is difficult to find a single cure.

Possible approaches being explored include strategies to:

- Prevent blood vessel formation and oxygen supply to tumors
- Turn off telomerase - the “fountain of youth” gene
- Induce immune response to tumors
Drugs are being designed to specifically treat subtypes of breast tumors that carry mutations that make them susceptible to specific drugs.

Two designer drugs are currently in use for breast cancer
Herceptin Therapy For HER2 Positive Tumors

HER2 antibody

Cell proliferation
Cell survival
Anti-Estrogen Therapy

Estrogen

Tamoxifen

Cell proliferation
Tumor Growth
Future Drugs

• Requires understanding of the pathways that drive the formation of different types of tumors

• Need to identify which proteins of the many that are altered play a primary role in the disease and develop strategies to inhibit these diseases.

• Initial successes in breast cancer and leukemias give hope for the future.
• Get students excited about what is happening in biology research

• Give them a chance to experience the thrill of discovery, no matter how trivial

• Contact congressman to support continued increases in NIH and NCI budget

Reducing cancer death by 20 percent would return $10 trillion dollars to the Americans!
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- Susan Komen Breast Cancer Foundation
- Leukemia Society
- National Cancer Institute

BREAST CANCER 1000
- Breast Cancer Research Foundation
- L’Oreal
- Private Donor

Web sites for materials for presentation
- NABCO - http://www.nabco.org
- NCI - http://www.cancernet.nci.nih.gov/
How do Our Genes Get Mutated?

- Some mutations are inherited
- Some occur spontaneously
- Some are caused by environmental factors
Inherited Mutations

BRCA-1

BRCA-2

Risk of getting breast cancer by age 70: 82%

Family history of premenopausal breast cancer
Non-genetic Factors that Influence the Incidence of Cancer

Risk factors that have been described:

- Never having given birth
- Late age at first pregnancy (after 30)
- Early onset of menstruation (<12)
- Late age onset of menopause (>55)
- Hormone replacement after menopause (longterm)
- Smoking
- Alcohol
- Obesity after menopause
- Lack of Exercise