Genomic Medicine and Breast Cancer
Past, Present, and Future

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Genomic Medicine and Breast Cancer

Genomic Medicine Definition

The use of molecular Genotype (DNA) and Phenotype (mRNA) to predict disease incidence, outcome, and/or to dictate treatment
Genomic Medicine and Breast Cancer

Genomic Medicine Definition

The use of molecular Genotype (DNA) and Phenotype (mRNA) to predict disease incidence, outcome, and/or to dictate treatment

In cancer biology there are two genomes

Tumor (somatic)

Patient (germline)
Genomic Medicine and Breast Cancer
Past

Treatment based on:

Clinical features of the tumor
(size, pathologic grade, nodal metastasis)

Expression and/or genetic abnormalities of one
or a few genes in the tumor
The First Therapy Based on Tumor Phenotype

Estrogen Receptor

DR. BEATSON: INOPERABLE CASES OF CARCINOMA OF THE MAMMA. [JULY 11, 1896.]

another thirty years it would then have entirely disappeared. The first great drop in its rate took place in the decade 1840-50, about the time that serious attention began to be given to sanitary reforms and especially to land drainage. It then remained scarcely reduced for about seventeen years; but from 1867 to 1894 it has been steadily on the decline. It is in this period that most of the great sanitary works have been carried out in this country. Can we doubt that it is to them that we owe so substantial a diminution of the disease? And need we despair of carrying it on to its fitting close? Let it be remembered that this improvement has taken place in spite of the increasing aggregation of the population in towns and without any special measures of repression having been attempted. It is, indeed, only recently that...
The First Therapy Based on Tumor Phenotype
Estrogen Receptor

Case History

33 year old premenopausal woman presented with a 11 X 8 cm left breast tumor with skin involvement

Patient underwent radical mastectomy

3 months later presented with diffuse chest wall and skin involvement with tumor and apparent metastatic disease in her thyroid

Patient underwent oophorectomy 1 month later and had a complete remission of her tumor and survived for 4 years
The First Therapy Based on Tumor Phenotype
Estrogen Receptor

Cytosol

Nucleus

mRNA

DNA

E2

ER

ER

ER

ER

ER

ER

ER
The First Therapy Based on Tumor Genotype
HER2/Neu amplification

Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene

Dennis J. Slamon,* Gary M. Clark, Steven G. Wong, Wendy J. Levin, Axel Ullrich, William L. McGuire

Slamon et al., Science 235: 177, 1987
The First Therapy Based on Tumor Genotype
HER2/Neu amplification

Slamon et al., Science 235: 177, 1987
The First Therapy Based on Tumor Genotype

HER2/Neu amplification

EGFR (HER-1, ERBB-1) (ERBB-2, Neu)
The First Therapy Based on Tumor Genotype
HER2/Neu amplification

EGFR/HER-2 Ligands
HER-3 Ligands
HER-4 Ligands

Proliferation
Anti-apoptosis
Invasion
Migration
Adhesion
Angiogenesis
Differentiation
Genomic Medicine and Breast Cancer Present

Treatment based on:

Clinical features of the tumor
(size, pathologic grade, nodal metastasis)

Expression and/or genetic abnormalities of multiple
genes in the tumor
  Estrogen Receptor and Progesterone Receptor
  HER2/Neu Amplification
  Recurrence Score
  Gene Expression Microarrays
Recurrence Score

Developed to stratify the risk of relapse and/or need for chemotherapy
   Early stage
   Hormone receptor positive tumors
   Node negative
   Tamoxifen treated

21 Gene set developed from literature and array experiments

Designed to use Quantitative Reverse Transcription PCR (qRT-PCR) of
RNA from formalin fixed paraffin-embedded tumor tissue

Recurrence Score
Quantitative Reverse Transcription PCR

mRNA 5' → 3'
Reverse Transcribe RNA

cDNA 3' ← 5'
Bind Molecular Probe

Molecular Probe

5' ← 3' ← 5'
Perform PCR

Probe cleavage and release of reporter

Multiple Rounds of PCR
## Recurrence Score
### mRNA Targets

<table>
<thead>
<tr>
<th>Proliferation</th>
<th>HER2</th>
<th>Invasion</th>
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</thead>
<tbody>
<tr>
<td>Ki-67</td>
<td>HER2</td>
<td>Stromolysin 3</td>
</tr>
<tr>
<td>STK15</td>
<td></td>
<td>Cathepsin L2</td>
</tr>
<tr>
<td>Survivin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclin B1</td>
<td></td>
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</tr>
<tr>
<td>MYBL2</td>
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<table>
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<tr>
<th>Estrogen</th>
<th>HER2</th>
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<tbody>
<tr>
<td>ER</td>
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</tr>
<tr>
<td>PR</td>
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<tr>
<td>Bcl2</td>
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<tr>
<td>SCUBE2</td>
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<table>
<thead>
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<th>HER2</th>
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<tbody>
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<td>GRB7</td>
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<th>Other</th>
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<tr>
<td>GSTM1</td>
</tr>
<tr>
<td>CD68</td>
</tr>
<tr>
<td>BAG1</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Reference</th>
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<tr>
<td>Beta-actin</td>
</tr>
<tr>
<td>GAPDH</td>
</tr>
<tr>
<td>RPLPO</td>
</tr>
<tr>
<td>GUS</td>
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<tr>
<td>TFRC</td>
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Recurrence Score

Continuous Predictor of Recurrence

Recurrence Score

Prognosis

<table>
<thead>
<tr>
<th>Risk</th>
<th>Score</th>
<th>% Pts</th>
<th>RR at 10y</th>
</tr>
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<tbody>
<tr>
<td>Low (338)</td>
<td>RS≤17</td>
<td>51</td>
<td>6.8</td>
</tr>
<tr>
<td>Intermediate (149)</td>
<td>RS 18-30</td>
<td>22</td>
<td>14.3</td>
</tr>
<tr>
<td>High (181)</td>
<td>RS≥31</td>
<td>27</td>
<td>30.5</td>
</tr>
</tbody>
</table>

Recurrence Score

Tumor Size

Paik et al., J Clin Oncol 24: 3726, 2006
Recurrence Score
Tumor Grade

Recurrence Score

Tumor Grade (Site)

Well

Moderate

Poor

N=77

N=339

N=163

12% 16% 73%

22% 22% 56%

42% 22% 36%

p<0.001

Paik et al., J Clin Oncol 24: 3726, 2006
## Recurrence Score
**HER2/Neu Amplification**

<table>
<thead>
<tr>
<th>Oncotype Risk Group</th>
<th>HER2/Neu Amplified</th>
<th>HER2/Neu Not Amplified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Risk</td>
<td>0</td>
<td>334</td>
<td>334</td>
</tr>
<tr>
<td>Intermediate Risk</td>
<td>5</td>
<td>142</td>
<td>147</td>
</tr>
<tr>
<td>High Risk</td>
<td>50</td>
<td>129</td>
<td>179</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>605</td>
<td>660</td>
</tr>
</tbody>
</table>
Recurrence Score
Node Positive Tumors

**Figure A**

- **Node Negative**
  - Low: 513 (59%) events 20
  - Int: 229 (26%) events 24
  - High: 130 (15%) events 28
  - Log-rank $P < .001$
  - RS Group: High v Low 5.2 (2.7 to 10.1)
  - HR 95% CI: 2.5 (1.3 to 4.5)

**Figure B**

- **Node Positive**
  - Low: 160 (52%) events 25
  - Int: 94 (31%) events 25
  - High: 52 (17%) events 24
  - Log-rank $P < .001$
  - RS Group: High v Low 2.7 (1.5 to 5.1)
  - HR 95% CI: 1.8 (1.0 to 3.2)
Recurrence Score and Chemotherapy
Node Negative Tumors

All Patients

Low Risk
RS ≤17

Intermediate Risk
RS 18-30

High Risk
RS ≥31

Paik et al., J Clin Onc 24: 3726, 2006
Gene Expression Microarrays
MammaPrint

Developed to predict risk of relapse in early stage patients
   Early stage
   Hormone receptor positive and negative
   Node positive and negative

70 gene set developed from cDNA microarray of ~25,000 genes

van’t Veer et al., Nature, 415, 530, 2002
Gene Expression Microarrays
Microarray Technology

A Creation of microarray

Defined oligonucleotides

1 2 3 4 5 6 7 8 9

Phoeto-lithography or other technique

DNA “chip”

1 2 3
4 5 6
7 8 9

B Differential hybridization

Sample DNA

1 2 3 4 5 6
7 8 9

Hybridization

1 2 3
4 5 6
7 8 9

Fluorescent label

C Detection

Patient 1

Patient 2

Patient 3

Patient 4

Gene Expression Microarrays
Mammaprint

Gene Expression Microarrays
Mammaprint

A  All Patients

B  All Patients

Probability of Remaining Metastasis-free

Good signature
Poor signature

Overall Survival

Good signature
Poor signature

P<0.001

Years

Gene Expression Microarrays
Mammaprint

C Lymph-Node–Negative Patients

D Lymph-Node–Negative Patients

E Lymph-Node–Positive Patients

F Lymph-Node–Positive Patients

Comparison of Molecular Classifications of Breast Cancer

D Recurrence Score

![Graph showing probability of overall survival over months for different recurrence scores.]

- High
- Intermediate
- Low

P < 0.001

21 genes

F 70-Gene Profile

![Graph showing probability of overall survival over months for different 70-gene profiles.]

- Good
- Poor

P < 0.001

70 genes

B Intrinsic Subtype

![Graph showing probability of overall survival over months for different intrinsic subtypes.]

- Basal-like
- HER2+ and ER-
- Luminal A
- Luminal B
- Normal-like

P < 0.001

476 genes

H Wound Response

![Graph showing probability of overall survival over months for different wound responses.]

- Activated
- Quiescent

P < 0.001

442 genes

Genomic Medicine and Breast Cancer Future

Treatment based on:

Clinical features of the tumor
(size, pathologic grade, nodal metastasis)

Expression and/or genetic abnormalities of multiple genes in the tumor
  Estrogen Receptor and Progesterone Receptor
  HER2/Neu Amplification
  Measures of multiple gene expression
  Pharmacogenomics
  Whole genome sequencing
Expression Profiling of Breast Cancer

Intrinsic subtypes

Molecular Profiling of Breast Cancer
Triple Negative Breast Cancer

Lehmann et al., J Clin Invest 121: 2767, 2011
Pharmacogenomics

Using genetic information (genotype or phenotype) to predict drug efficacy or toxicity
Pharmacogenomics

Using genetic information (genotype or phenotype) to predict drug efficacy or toxicity

In Cancer Biology there are two genomes

Tumor (somatic)

Patient (germline)
Pharmacogenomics

Tumor Pharmacogenomics:

Presence of the therapeutic target predicts treatment benefit

Estrogen Receptor

HER2/Neu amplification
Tumor Pharmacogenomics:

Presence of the therapeutic target predicts treatment benefit

- Estrogen Receptor → Anti-hormonal agents
- HER2/Neu amplification → Anti-HER2/Neu agents
Tumor Pharmacogenomics:

Genetic abnormality that predicts a treatment benefit

BRCA1 and BRCA2 mutations
Pharmacogenomics

DNA damage
Double Strand DNA Breaks

Homologous Recombination
BRCA1 and BRCA2

Alternative Repair
e.g., PARP

DNA Repaired
Pharmacogenomics

DNA damage
Double Strand DNA Breaks

Homologous Recombination
BRCA1 and BRCA2

X

Alternative Repair
e.g., PARP

DNA Repaired
Pharmacogenomics

- DNA damage
- Double Strand DNA Breaks
- Homologous Recombination
  - BRCA1 and BRCA2
- Alternative Repair
  - e.g., PARP
- PARP inhibitors
- Cell Death
Pharmacogenomics

Patient Pharmacogenomics:

Presence of genotypic or phenotypic markers in the patient that predict a drug's efficacy or toxicity
Patient Pharmacogenomics
Phenotype and Hormonal Treatment

Hypothalamus

\[ \text{LHRH} \]

\[ \downarrow \]

Pituitary

\[ \text{LH} \]
\[ \text{FSH} \]

\[ \downarrow \]

Ovary

\[ \text{E2} \]

= Breast Cancer

\[ \text{ER} \]

Adrenal Gland

\[ \text{A} \]

\[ \downarrow \]

Fat
Skin
Breast Cancer

\[ \text{Aromatase} \]

\[ \downarrow \]

\[ \text{E2} \]
Patient Pharmacogenomics
Phenotype and Hormonal Treatment

Hypothalamus
  ↓
  LHRH

Adrenal Gland
  ↓
  A
  ↓
  Aromatase
  ↓
  Fat
  Skin
  Breast Cancer

Pituitary
  ↓
  LH
  ↓
  FSH

Ovary
  ↓
  E2

Breast Cancer
  ↓
  ER

Both

Premenopausal Women
  ↓
  E2

Postmenopausal Women
Patient Pharmacogenomics:

Presence of genotypic or phenotypic markers in the patient that predict a drug's efficacy

Metabolic enzyme isotypes may affect metabolism of drugs
e.g., cytochrome p450 enzymes

Most commonly single nucleotide polymorphisms (SNPs)
Genomic Sequence Variation
Single Nucleotide Polymorphism (SNP) Arrays

Genomic Sequence Variation
Whole Genome Sequencing

DNA sample
Fragment sample

Traditional sequencing
Clone fragments into bacteria and select clones
Grow bacteria, isolate DNA
Create multiple copies of single fragment
Sequence about 100 fragments in parallel (≈1 Kb each)
0.1 Mb of sequence per “run”

Next-generation sequencing
Separate fragments in fluid or solid substrate
With or without PCR amplification
Create multiple copies of multiple fragments
Sequence millions of fragments in parallel (50 to 100 bp each)
>100 Gb of sequence per “run”
Genomic Sequence Variation
Whole Genome Sequencing

Genomic Sequence Variation
Whole Genome Sequencing
PI3K Pathway Mutations

Genomic Medicine and Breast Cancer
Past, Present, and Future

Prognostic determination and treatment decisions

Past: Tumor characteristics (size, grade, nodal metastasis)
  Expression or mutation of a few genes
    e.g., ER, PR, HER2/Neu

Present: Tumor characteristics (size, grade, nodal metastasis)
  Expression or mutation of a multiple genes primarily in the tumor
    e.g., Recurrence Score, Microarrays

Future: Tumor characteristics (size, grade, nodal metastasis)
  Expression or mutation of many (perhaps hundreds) of genes in the tumor and the patient
    e.g., Whole genome sequencing, SNP arrays